The behaviour and wing morphology of the meadow brown butterfly (Maniola jurtina L.) in Britain: the influence of weather and location

Celia Maier (1998)

THE BEHAVIOUR AND WING MORPHOLOGY OF THE MEADOW BROWN BUTTERFLY (*MANIOLA JURTINA* L.) IN BRITAIN: THE INFLUENCE OF WEATHER AND LOCATION

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British Butterflies. W.S. Coleman, 1868

"THE MEADOW BROWN BUTTERFLY
(*Hipparchia Janira)*

Perhaps of all our butterflies this is the least attractive, being too common to excite interest from its rarity or difficulty of attainment, and too plain and homely to win regard. This is the sober brown insect that keeps up a constant fluttering, in sunshine and gloom, over the dry pasture land and barren hill-side; and perhaps it ought to find favour in our eyes, from this very fact of keeping up a cheerful spirit under circumstances the most unfavourable to butterfly enjoyment in general."

"I learned new things about this common butterfly. I also learned something new about myself, something partly forgotten over the long winter."

Butterfly Watchers. Robert Michael Pyle, 1984

ABSTRACT

Understanding the influence of climate and weather on butterfly abundance and distribution, as well as the morphological, physiological and life-history traits associated with populations living in different geographic and climatic areas, is critical to the consideration of how they respond to environmental change. Using *Maniola jurtina* as a model species, research was conducted to determine the effect of meteorological variables on the behaviour of this species in south central England and north west Scotland, and to determine whether variation in wing morphology is adaptive in terms of thermoregulatory efficiency. Temperature, solar radiation and wind speed were recorded simultaneously with timed behavioural observations, which were made on transect walks and whilst following individual butterflies. Thoracic temperatures were recorded in the field, and wing size and darkness determined using digital image analysis. Warm-up rates of butterflies of known wing morphology were determined under laboratory conditions. In the north, conditions are cooler, cloudier and windier; habitat is more restricted and butterflies were found to be at lower density than in the south. Temperature and solar radiation intensity influenced duration of flight, feeding and basking (butterflies in both regions used both dorsal and lateral basking postures), with the largest effects being shown for the southern population. Males from both regions flew for significantly longer than females and northern males flew for significantly longer than those from the south. Northern butterflies are larger and darker than those from the south, but there is no significant difference in thoracic temperatures between the regions. Smaller, darker butterflies were active at lower air temperature and solar radiation intensity than larger, paler individuals. Although northern butterflies show morphological and behavioural adaptations to marginal weather conditions and low population density, a model of egg production estimates that fecundity of northern females is reduced by 16% compared to those in the south.

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ABBREVIATIONS

T_b	butterfly body temperature (°C)
T_{ex}	thoracic temperature excess (°C) (thoracic temperature minus ambient air temperature)
T_{th}	thoracic temperature (°C)
T_a	ambient air temperature (°C)
T2	air temperature at height of ≅ 80 cm (°C)
T3	ground temperature (°C)
SR	solar radiation intensity (Wm^{-2})
WS	wind speed ($m\ min^{-1}$)
DB	dorsal basking posture
LB	lateral basking posture
B	basking (dorsal and lateral) posture
I	intermediate posture (alighted, neither basking nor heat avoiding)
A	heat avoiding posture
FWD	flew when disturbed (escape flight)
BGV	basal grey value (darkness of wing, on a scale of 0 = black to 255 = white)
PGI	phosphoglucose isomerase
PGM	phosphoglucose mutase

CHAPTER 1. INTRODUCTION

Maniola jurtina (L) began its scientific career in confusion. The generic name *Maniola* has been variously linked to "the dusky souls of the departed" or a "hell-born manic frenzy". The generic name *jurtina* probably arose from a typographical error from Jurturna (the name of a nymph). Linnaeus originally referred to *Jurtina* for only the male meadow brown, a grassland species, while classifying the female as a separate species, *Janira,* which inhabited woodland (Emmet 1991).

Despite this initial confusion, *Maniola jurtina* has become one of the most intensively studied species of butterflies. Its role in ecological genetics has been reviewed by Ford (1975), Dowdeswell (1981) & Brakefield (1984). The variation and functional significance of its hind wing spots has also been extensively studied by Brakefield (1979) (but see Shreeve, Dennis & Williams 1996). Phenotypic variation is reviewed by Emmet & Heath (1989) and includes studies by Thomson (1971), Tudor & Parkin (1979) and Goulson (1991). Studies on the ecology of *M. jurtina* include Pollard (1981), Brakefield (1982a, 1982b), Shreeve (1989), Goulson (1991) and Feber (1993). *M. jurtina* is both common and widespread throughout most of the British Isles (Emmet & Heath 1989). This, together with the wealth of information already available on the species, makes it an excellent model for research, which aims to elucidate wider ecological principles.

In recent years, with the spectre of global warming, there has been increasing interest in the influence of climate and weather on butterfly abundance and distribution, as well as the morphological, physiological and life-history traits associated with populations living in different geographic and climatic areas (Dennis & Williams 1986, Turner *et al.* 1987, Dennis & Shreeve 1989, Dennis & Shreeve 1991, Dennis 1993, Pollard & Yates 1993, Bryant *et al.* 1997). Butterflies living at the edge of their range may be close to the limit of their physiological tolerances, and this, together with demographic factors and limits on availability of suitable habitat, is likely to make them more vulnerable to variation in meteorological conditions (Shreeve *et al.* 1996). Adverse weather conditions are likely to have an impact on abundance and density of butterflies, and the

low density of butterflies in marginal populations may, in turn, influence activities such as mate location behaviour, which could have a direct effect on reproductive success.

Weather is a major factor influencing the success of individual butterflies, through its effect on their ability to carry out all the activities required for survival and reproduction (mate location, feeding, egg-laying and predator avoidance). The success of individuals translates into differences in population size and dynamics in different areas of the species range. Any attempt to understand and predict the effect of changes in weather conditions must include an analysis of how variation in meteorological variables, such as temperature, solar radiation and wind, influence individuals' behaviour in different parts of a species range. Adaptations, such as variation in wing morphology, which ameliorate the effects of marginal weather conditions are also of interest here.

Research in North America, particularly that by Ward Watt and Joel Kingsolver, has produced a large body of literature on the influence of weather conditions on flight activity and reproductive success of *Colias* butterflies. This American work has also examined the adaptive significance of variation in wing morphology in terms of thermoregulation, for butterflies living at different altitudes. In Britain there are few detailed studies of the influence of weather on butterfly behaviour on a "micro-scale", or on the adaptive significance of variation in wing morphology of British butterflies. Here, I have used meadow browns to try and address some of these issues.

Shreeve *et al.* (1996) state that "Much can be discerned, we believe, from direct comparisons between core and peripheral populations...". In this research I use *Maniola jurtina* as a model for investigating variation in behaviour and aspects of wing morphology in different areas of the species range (south-central England and north-west Scotland), and discuss the adaptive significance of such variation. I also make use of detailed environmental data (temperature, solar radiation and wind speed), measured on a time and spatial scale appropriate to the changes in butterfly activity, to determine how behaviour is influenced by meteorological variation at this scale.

1.1 Aims

Using *M. jurtina* as a model species, the primary aims of my work were:

1. To determine the specific effect of the temperature, solar radiation and wind speed on the behaviour of meadow browns.

2. To investigate whether there are differences in behaviour between male and female butterflies and between different geographic/climatic areas.

3. To determine whether variation in wing morphology within populations and between populations in different geographic/climatic areas is adaptive in terms of thermoregulatory efficiency.

Subsequent chapters give specific objectives concerning each area of research, which was used to address these general aims.

1.2 Structure of the thesis

In the following chapter, I review literature on wing morphology and behaviour, to place my research in a wider context. I then give a summary of the current knowledge relating to the ecology of meadow browns in Britain. In the subsequent chapter, I describe the general methods used in this research.

Mean July temperatures have been shown to correlate with species abundance and diversity, so I give an overview of general climate and weather conditions in the two regions used in this study; north west Scotland and south central England. Density of butterflies is influenced by many factors, including weather and habitat, and density may in turn influence behaviour and population dynamics, so I include a comparison of the density of meadow browns in the two study areas. The chapters on behaviour begin with an overview of how behaviour in the two regions varies with weather conditions, to provide a framework for later more detailed analyses. Each category of behaviour is subsequently analysed in detail, in relation to environmental variables, with comparisons between males and females and between the southern and northern regions.

The next two chapters give the results of investigations into thoracic temperatures in the field and the relationship between wing morphology and thermoregulation. Finally, I discuss the main results of this study and their significance, and make suggestions for future lines of research.

CHAPTER 2. LITERATURE REVIEW

2.1 INTRODUCTION

Butterfly wing morphology (size, shape, colour and pattern) is important in determining individual success, through its influence on thermoregulation, mate acquisition and predator avoidance. The different components of wing morphology fulfil different functions: size and shape can affect predator avoidance strategy (Chai & Srygley 1990), mate location strategy (Wickman 1992), and thermal stability (Heinrich 1993). Colour, but probably not fine pattern detail, is important in intra-specific communication (Silberglied 1984), whereas pattern detail is used for primary and secondary defence (Endler 1978). Melanization is of prime importance in thermoregulation and thermal stability, which in turn influences activity patterns (Watt 1968, Kingsolver 1985a).

Butterfly wing patterns are made up of discrete elements, organised in several symmetry systems, which are arranged across the wing, parallel to the outer margin. Pattern formation is further compartmentalised by the wing veins (which run perpendicular to the symmetry systems). The resulting pattern elements, (compartmentalised by both symmetry systems and wing veins) can vary and evolve independently of the other pattern elements (Nijhout 1994). The flexibility and "mosaic" nature of this pattern development system helps explain the enormous diversity and divergence found in butterfly wing patterns. The form of pattern elements is determined by the deposition of pigments in the individual wing scales. The resultant arrangement of colours and shapes across the wing surfaces allows the wings to fulfil the different functions of thermoregulation, predator avoidance, communication and mate acquisition - and is strongly influenced by the requirements of these different functions (Dennis & Shreeve 1989).

The allocation of different functions to each wing surface is dependent on the posture adopted by the resting but potentially active individual. The posture adopted is conservative within taxonomic groups. The resultant wing morphology can be

described as a compromise between the requirements for the different functions that it has to fulfil (Dennis & Shreeve 1988, 1989, Shreeve & Dennis 1992).

Most species of butterfly are associated with specific habitat types. Within any particular environment there will be constraints which influence activity and the chance of encountering mates and avoiding predators. Where thermal conditions are marginal for activity, morphological adjustments that increase thermal efficiency are of prime importance (Roland 1982, Guppy 1986a, 1986b) as are those for primary defence and crypsis. However, low population density, which is characteristic of marginal locations, may increase the need for greater apparency which in turn may compromise predator avoidance (Dennis & Shreeve 1989, Shreeve & Dennis 1992, Dennis 1993). In conditions more favourable for activity, the balance of selective pressures on overall wing morphology will differ (e.g. butterflies may spend a greater proportion of the time in flight, so secondary defence will be of greater significance).

2.2 THERMOREGULATION

Flight is the cornerstone of butterfly behaviour, but cannot take place until thoracic flight muscles reach a threshold temperature. This temperature is approximately 28-30° C for the majority of species studied to date (exceptions are some of the slower flying species such as *Danaus plexippus* and *Phoebus* spp., which can fly at a lower thoracic temperature - Chaplin & Wells 1982, Guppy 1986b). The importance of reaching this threshold thoracic temperature for flight puts thermoregulation at the core of butterfly ecology.

In temperate regions, butterflies generally need to achieve thoracic temperatures several degrees higher than ambient air temperature (T_a) before the thoracic muscles are warm enough for active flight. During pre-flight warm up, butterflies are usually ectothermic (acquiring heat from the environment to raise their body temperature). When roosting, all are poikilothermic (variable body temperature that is dependent on the external environment). Active flight is essential for butterfly survival and reproduction, so the weather, particularly temperature and solar radiation, is a key influence on butterfly behaviour and population dynamics.

2.2.1 Thermoregulation and Flight

Individual Success & Population Dynamics

Flight is essential for locating mates, food resources and egg laying sites; for escaping predators and for dispersal. Kingsolver (1985a) argues that thermoregulation and flight activity, through their effects on individual success in terms of realised fecundity, are the most important link connecting weather (principally temperature, solar radiation and wind speed) to the population ecology and dynamics of temperate butterflies. This argument draws on earlier work, such as that of Dempster (1983), who used key factor analysis in a review of population fluctuations in temperate zone Lepidoptera. Dempster concluded that the two most important factors influencing fluctuations in abundance from one generation to the next were predation and failure of females to lay their full complement of eggs.

Empirical work supports the view that the influence of weather on flight activity and individual success, may be expressed through its effects on both survivorship and fecundity.

Survivorship & Predation

Active flight is a key mechanism for escaping predation. Several studies suggest that butterflies are most at risk in marginal temperature and weather conditions when effective thermoregulation and active flight are difficult. Bowers *et al.* (1985) found that a high frequency of beak marks on *Euphydryas chalcedona*, indicating increased predation, was associated with unusually cold and cloudy weather. Rawlins & Lederhouse (1978), and Lederhouse (1983) found that there was a weather related increase in mortality in the black swallowtail, *Papilio polyxenes,* associated with roosting under low temperature conditions. Kingsolver (1987b) found that predation on *Colias* and *Pieris* species was greatest in the cooler early morning pre-flight period.

Fecundity

Weather conditions and the ability to thermoregulate effectively can influence fecundity by affecting the rate at which eggs mature in species where females emerge without a full complement of mature eggs (e.g. Stern & Smith 1960, Gossard & Jones 1977).

They also affect flight time and hence time available to locate suitable egg laying sites. Courtney (1984), summarised field data for Lepidoptera that lay eggs singly and found that mean realised fecundity was less than one third of the potential maximum. Kingsolver (1983b) estimated that female *Colias* at high elevations could lay only 20 - 50% of their full egg complement because of limited flight time. Watt (1968) also found that egg production was reduced in unfed *Colias* females - with reduced feeding rates being another consequence of decreased flight time.

Although low temperatures are often cited as a major contributing factor to increased risk of predation and decreased egg production, overheating can also have a significant impact by reducing flight time, survival and fecundity (Rawlins 1980, Kingsolver & Watt 1983, 1984, Heinrich 1993). Intermittent overheating in males may also force them to abandon their defended mating territories in territorial species (Lederhouse 1982) and reduce the time available for mate searching in patrolling species (Roland 1982).

2.2.2 Flight temperatures

Most butterflies can fly when thoracic temperatures (T_{th}) are in the range of approximately 28-40° C, with vigorous flight restricted to a range of 33-38° C (Kingsolver 1985a). Several north American *Colias* species have been found to initiate flight when thoracic temperatures reach approximately 30° C, with most vigorous flight at 35-38° C. They first show heat avoidance behaviour and then cessation of flight when T_{th} reaches 40-42° C. (Watt 1968, Kingsolver 1983a, 1983b, Kingsolver & Watt 1984). Similar T_{th} for flight initiation have been found for other temperate species which fly with a moderate to fast wing-beat frequency e.g. 30° C for *Colias nastes* (Roland 1982), 32-34° C for *Pararge aegeria* (Shreeve 1984), 28-30° C for *Papilio machaon* (Wasserthal 1975), 29-30° C for *Pieris* spp. (Kingsolver 1985c). Heinrich (1993), however, criticises the work of Kingsolver and Watt and argues that the temperatures given for flight initiation and vigorous flight in *Colias* may be artificially high. He reports that minimum temperatures for flight in *Colias* are 19 - 20 °C (rather than 28 °C) and that overheating may occur at thoracic temperatures of ≥ 34 °C. Watt (1997) rejects

these criticisms, suggesting that evidence cited by Heinrich is anecdotal and insufficiently rigorous.

For species with slow, gliding flight, which do not require the same power and frequency of muscle contraction, flight may be initiated at lower thoracic temperatures; e.g. 10° C for overwintering monarch butterflies, *Danaus plexippus,* (Chaplin & Wells 1982) and 17-18° C for *Parnassius phoebus* (Guppy 1986b).

2.2.3 Mechanisms of Thermoregulation

A controlled body temperature can be achieved and maintained by regulating either heat gain or heat loss. Both these features can be found in butterfly thermoregulation. There are two possible options for heat gain during pre-flight warm-up; physiological (generating internal metabolic heat) or behavioural (choice of microhabitats, orientation and posture relative to the sun) (Heinrich 1981, 1993). Heat loss can be affected by a number of factors including morphological characteristics such as size and pubescence. *Colias,* for example, in cool, windy environments are hairier than those in warmer, less exposed sites (Kingsolver & Moffat 1982), with hair on the ventral thorax reducing heat loss by as much as 70%.

2.2.3.1 Physiological Mechanisms of thermoregulation

During flight, rapid contraction of the flight muscles generates heat. Many large moths, such as some of the Sphingids and Saturniids, can generate thoracic temperatures 10-20° C above ambient (excess thoracic temperature, T_{ex}) by this means (e.g. Heinrich 1971, Heinrich & Casey 1973). For butterflies, however, heat generated during flight does not always lead to increased thoracic temperature. Increased thoracic temperature during flight (in the range of 3-6° C), under conditions of low solar radiation, has been demonstrated in a few species of butterflies, e.g. *Danaus plexippus* (Kammer 1970) and *Papilio polyxenes* (Rawlins 1980). In other species, temperature may remain stable during flight e.g. *Colias* (Tsuji *et al.* 1986), *Parnassius phoebus* (Guppy 1986b). Several studies have also noted a drop in temperature during flight for some species, e.g. *Coenonympha inornata* (Heinrich 1986a), *Pararge aegeria* (Shreeve 1984) and *Calisto nubila* (Shelly & Ludwig 1985). Most of the heat loss during flight is due to

convection. Whether or not there is a net gain in body temperature during flight will depend to a large extent on wing beat amplitude and frequency. These in turn have a relationship with both the temperature of flight muscles and disturbance of the boundary layer of air around the wing, and hence convective heat loss.

So, for butterflies, heat generated by physiological mechanisms during flight may or may not be of significance for thermoregulation, depending on the species. Another means of generating heat from the flight muscles is through rapid contraction of the elevator and depressor flight muscles against each other (rather than the wings), leading to high frequency, small amplitude wing movements known as shivering (or wing whirr). This means of pre-flight warm is well documented for many nocturnal moths (e.g. Adams & Heath 1964, Heath & Adams 1967, Kammer 1968, Heinrich 1971, Heinrich 1993). Shivering has been reported in a number of species of butterflies, including members of the families Papilionidae, Hesperidae, Nymphalidae and Pieridae (e.g. Findlay, Young & Findlay 1983, Dennis 1993, Schmitz 1994, Maier & Shreeve 1996). Primary data on direct observations of shivering, although infrequent, are suggestive that this means of pre-flight warm-up may be a normal part of the behavioural repertoire of some species.

There are a few instances where this means of pre-flight warm up has been studied in detail. Kammer (1970) found shivering was used by monarch butterflies, *Danaus plexippus*, at low temperatures (T_a of 13° C) and that they could raise their thoracic temperature by 4° C, at a rate of 1.3° C min^{-1}. The black swallowtail, *Papilio polyxenes,* uses shivering as a subsidiary mechanism under conditions of low solar radiation and ambient temperature. Under these circumstances shivering probably generates enough heat to allow minimal activity for activities such as changing habitat or regaining a roosting site if dislodged (Rawlins & Lederhouse 1978). Srygley (1994) describes shivering in two species of Neotropical owl butterflies *Caligo* and *Opsiphanes*, which could achieve thoracic temperatures of 10°C and 15°C above ambient air temperature respectively. These butterflies are crepuscular, flying at dawn and dusk, when using solar energy through basking is not an option. The minimum ambient air temperature recorded in the field was 24°C, which was 4 - 5°C above the minimum temperature

required for flight. Srygley suggests that the primary function of shivering in these butterflies was to elevate thoracic temperature to increase flight performance, and hence success in reproductive encounters.

Excess thoracic temperatures of up to 20°C, following prolonged shivering, are not uncommon in many species of moths, chiefly Sphingidae and Saturnidae (e.g. Heinrich & Bartholomew 1971, Bartholomew & Casey 1973). Comparable excess thoracic temperatures achieved through shivering have been recorded for several species of Nymphalidae: 18°C in *Vanessa atalanta* (Krogh & Zeuthens 1941); 8 - 11°C in *Nymphalis antiopa* (Douglas 1986); 16.5°C, 18°C and 13°C in *Cynthia cardui*, *Inachis io* and *Vanessa atalanta* respectively (Maier & Shreeve 1996).

Most authors have concluded that endothermic shivering, as a means of pre-flight warm up in butterflies, is only a very minor thermoregulatory mechanism and is not of general significance (e.g. Kingsolver 1985a, Dennis 1993, Heinrich 1993). Shivering is energetically expensive, and when heat can be obtained from solar radiation this obviously requires less fuel and would be a more efficient way of raising body temperature.

Although it is possible that endothermy is an under-rated mechanism of thermoregulation in butterflies - at least for some species under some conditions - it is still the case that the major mechanisms for thermoregulation for all temperate species are behavioural.

2.2.3.2 Behavioural Mechanisms of Thermoregulation

The main way in which butterflies regulate their body temperature (T_b) is through adjustment of their posture and orientation in relation to the sun (for reviews see Dennis 1993, Kingsolver 1985a, Shreeve 1992). These adjustments will take place in the context of a particular microhabitat and its associated microclimate, so choice of sites for basking (or heat avoiding) is another important component of thermoregulatory activity (Casey 1981). *Pararge aegeria*, for example, uses hot, reflective sites (such as stones and bare earth) in woodland rides when basking early in the morning. As

temperatures increase during the day it moves to cooler areas under the canopy (Shreeve 1984). Rutowski *et al.* (1994) found that the position and height of perch sites chosen by *Asterocampa leila* changed throughout the day, depending on ambient air temperature.

2.2.3.3 Basking posture & orientation

Wings and thermosensitivity

It is now widely accepted that butterflies use their wings as devices to transform energy from solar radiation to heat, to raise the temperature of flight muscles in the thorax. Some of the early experiments, however, which compared temperatures and warm up rates in butterflies with and without wings, seemed to indicate that wings *per se* played no significant role in raising T_b in irradiated butterflies (Heinrich 1972, Kammer & Bracchi 1973). These experiments were criticised for using a flawed and inadequate design which invalidated the results (Wasserthal 1975). A large body of subsequent work has confirmed the importance of wings in regulating butterfly body temperature (e.g. Watt 1968, 1969, Wasserthal 1975, Kingsolver 1985c, 1987a). Wasserthal (1975), for example, demonstrated that in basking butterflies (comparing butterflies with wings shaded or irradiated), although direct heating of the body made the largest contribution to the temperature excess, wings contributed between 38% and 45% of the total body temperature excess (for *Apatura ilia* and *Papilio machaon* respectively).

When basking, the greatest heat gain is realised when the wings are orientated to intercept the maximum amount of solar radiation, i.e. perpendicular to the sun's rays. How the butterfly orientates its body to achieve this depends on the angle of the wings to each other. Clench (1966) recognised two main methods of basking; lateral (wings closed) and dorsal (wings open). He also described several subsidiary methods of thermoregulation. These methods included "ground contact" (now usually included as part of the behavioural repertoire of dorsal baskers) and "body basking" (wings held slightly open so that only the body is involved in heat absorption, as in *Pieris rapae*). The term "body basking" and the posture to which it was applied, have been reinterpreted in more recent work (e.g. Kingsolver 1985a, 1985b, 1985c - see below). Clench proposed that haemolymph flow through the wing veins could facilitate heat

transfer to the thorax, with the distal portions of the wings being of particular importance in this process. Subsequent studies disproved this idea, showing that haemolymph flow through the wing veins was too slow to have any significant effect on thoracic heating, and that there was no significant difference between warming rates of live and freshly dead butterflies (Watt 1968, Wasserthal 1975).

Schmitz & Wasserthal (1993), using dorsal basking Papilionid butterflies (*Troides* and *Pachliopta* species), identified sensory cells within the antennal clubs and wing veins which acted as thermoreceptor systems. Differences in mass and physical characteristics of the antennal clubs, wings and body lead to different warming rates and equilibrium temperatures when the butterfly is exposed to solar radiation during basking. The low mass, physical characteristics and effect of convective cooling meant that the temperature of the antennal clubs remained close to that of ambient air temperature during basking (maximum difference of + 4°C). The basal wing veins absorbed much of the solar radiation and were less subject to convective cooling, so heated at a greater rate and achieved equilibrium temperatures approximately 18°C higher than that of the antennae when fully exposed. Thoracic temperature and warming rate was intermediate between that of the antennae and wings.

Scanning the wing veins with a narrow, directional light beam elicited a slow wing closing response. The response appeared to be dependant on the steepness of the heating rate, so that the wings were closed and a heat avoiding posture adopted before temperatures had reached a critical level for overheating the wings or thorax.

Schmitz & Wasserthal suggest that the opening and closing of wings which occurs before a "settled" posture is adopted (characteristic of many dorsal basking Nymphalids), functions to monitor radiant intensity and hence determine the optimum wing angle and orientation. Schmitz (1994) also suggests that the pteral (wing) thermosensitive system, together with the visual system, is involved in orientation behaviour. Reducing the temperature difference between the right and left wings to zero would enable the butterfly to adopt a finely tuned basking position in relation to incoming solar radiation.

One can also speculate that the existence of separate thermoreceptive systems which appear to respond differentially to ambient air temperature and radiant energy (antennal and pteral respectively) would allow a more finely tuned response to these two environmental variables. This might allow the butterfly to make "better informed" behavioural adjustments (in terms of posture, orientation and choice of microclimate) not only along the "cool/cloudy" vs. "hot/sunny" weather axis, but also when conditions were either "warm but overcast" or "cool but bright".

Posture and heat transfer

In dorsal and lateral absorption baskers, the main method of heat transfer from wings to thorax is probably by conduction through the wing cuticle and veins. However, as the cuticle is thin and a poor conductor, only the portion of the wing nearest the body (the basal 5 - 10 mm) is involved in this process (Watt 1968, Wasserthal 1975, Kingsolver & Moffat 1982). Other methods of heat transfer include conduction of heat from a warm substrate and reducing convective currents by trapping a layer of warm air around the body.

In lateral baskers, which include Theclinae, Coliadinae and some Satyrinae, the wings are always held closed during basking. Heat is conducted from the basal ventral wing surfaces to the thorax and abdomen. Lateral basking posture, orientation and thermal ecology has been most extensively studied in N. American *Colias* spp. (e.g. Watt 1968, 1969, Hoffman 1974, Kingsolver & Moffat 1982, Kingsolver 1983a, 1983b, Kingsolver & Watt 1983, 1984,). In *Colias*, the degree of melanization (dark colouration) of the basal portion of the ventral wing surface has been shown to be an important variable effecting efficiency in absorption of solar energy and hence thermoregulation (see below). Some laterally basking species, such as *Gonepteryx rhamni* (the Brimstone), lack the dark wing bases found in *Colias*. Schmitz (1994) suggests that for *Gonepteryx* trapping warm air between the wings to conserve body heat and minimize convective heat loss is of prime importance. The light wing colour may also be advantageous in reducing radiative heat loss.

Findlay, Young & Findlay (1983) demonstrated the importance of fine adjustments in posture in relation to thermoregulation in the lateral basking butterfly *Hipparchia semele.* Both orientation and tilt can vary depending on body temperature and solar radiation, so that the area of wing exposed to solar radiation (and hence amount of radiation absorbed) varies. Body orientation relative to the sun, for example, changes from perpendicular to random to parallel as body temperature increases. Similar postural adjustments in *H. semele,* in response to increasing air temperature, are described by Dreisig (1995); termed "basking, graded and heat avoidance" phases.

In dorsal absorption baskers, which include most Nymphalidae, Hesperidae and some Lycaenidae (Shreeve 1992), the wings are held open. Heat is conducted from the basal portion of the dorsal wings to the thorax. Wing angle and body orientation may also be varied to expose greater or lesser areas of wing to the sun and to alter air flow around the wings, effecting heat loss by convective cooling. Maximum heat gain would be achieved with wings fully open, or in some cases pressed down against the substrate to trap warm air (e.g. Kevan & Shorthouse 1970) and orientated towards the sun. As body temperatures increase, the wings may be raised until a heat avoiding posture is adopted - wings closed and orientated parallel to the sun, as in heat avoiding lateral baskers.

Kingsolver describes two further methods of basking: "body basking", is used by several broad bodied butterflies such as skippers, where the two pairs of wings are held at an angle to one another and solar radiation is absorbed directly by the thorax (Kingsolver 1985a). He also describes a "new" method of basking, termed "dorsal reflectance" basking, found in *Pieris* spp. (Kingsolver 1985b, 1985c). The wings are held at an angle, which depends on wing size and pigmentation. Incoming solar radiation is reflected from the wing surface directly onto the thorax. In this posture the whole wing surface, rather than just the basal area, is relevant to thermoregulation. In order to reduce the amount of radiation reflected, wings may be either further opened *or* closed (unlike adjustments found in absorbence baskers). Kingsolver suggests that this mechanism is a derived posture, found only in *Pieris* spp. Shreeve (1992) suggests that it is also found in the Lycaenidae.

The existence of reflectance basking is not universally accepted. Heinrich (1990) found no evidence of the phenomenon in butterflies positioned with their wings at different reflective angles, and suggested that the "angled wing" posture observed in the field in many Pierids and Lycaenids, has more to do with varying air flow, and hence convective cooling, around the wings than with reflecting solar radiation. This experiment might be criticised as it used *Colias* (normally lateral basking) rather than *Pieris* species. Although the yellow colour of *Colias* and the white of *Pieris* are both derived from pteridine pigments, the reflectivity of the yellow *Colias* wing surface is only approximately half that of the white, glossy *Pieris*, which reflects over 80% on incoming solar radiation (Kingsolver 1985b).

In an extensive review of insect thermoregulation, Heinrich (1993) repeats his doubts about the existence of "reflectance" basking in Pierids. Schmitz (1994) also notes that Heinrich's use of the lateral basking *Colias* may not provide a valid model to refute the reflectance basking hypothesis. However, Schmitz also presents evidence that the wings of Pierids cannot act in the way suggested by Kingsolver (i.e. reflecting solar radiation onto the thorax in a directional, mirror-like fashion), as the rough irregular wing surfaces can only lead to diffuse reflectance. In spite of this problem with Kingsolver's reflectance basking model, Schmitz suggests that a modified version of the model may still be valid. The diffuse reflectance of the raised wings of Pierids could still serve to trap radiation and elevate thoracic temperatures - in addition to reducing heat loss through convective cooling.

2.2.4 Pigments and Thermoregulation

The colours of butterfly wings are due to properties of the wing scales. Colours may be either structural, pigment or a combination of the two (Nijhout 1991). Structural colours (e.g. the iridescent blues), are created by extremely fine physical structural details of the scales, which leads to scattering and interference patterns in the reflected light. Pigment colours are derived from four main groups of pigments; melanins (mainly black and brown, but also yellow and red); pterins (mainly whites and yellows through to red); flavonoids (white and yellow) and ommochromes (brown, red and yellow). Each scale is generally monochrome, so the overall colours and patterns of the

wings are the result of different arrangements and proportions of these pigment bearing cells, and (for iridescent colours) reflectance of light from the vanes, microribs and lamina of the scales (Nijhout 1991).

The pigments involved have different properties of absorption and reflectance, so wing pigmentation - both kind and degree - is a critical variable in thermoregulatory efficiency. In *Colias,* for example, black melanic regions of the wing absorb approximately 70% in the incoming radiation in the 400 - 1500 nm wavelength range (visible and near-infrared), while the yellow pteridine areas absorb only approximately 40% (Watt 1968, Kingsolver 1983b). Schmitz (1994) found that the black melanic wings of *Pachliopta aristolochiae* absorbed more than 96% of incoming radiation across the whole spectrum of visible light. The high absorbance of the wing was due to both pigmentation and to the microstructure of the scales, which almost entirely suppressed reflectance.

For efficient thermoregulation, reflectance baskers rely on a highly reflective wing surface, such as the glossy white pteridine pigments leucopterin and isoxanthapterin found in *Pieris* (Watt 1967). The reflective structural colours of many of the brightly coloured blues may also function in a similar way (but see above for discussion of reflectance basking). Absorbence baskers, both lateral and dorsal, rely on the opposite effect, with pigments such as melanin, which is common in nymphalids and satyrids, being used to absorb solar radiation. Although in temperate regions black and brown pigmentation is usually melanin derived and hence good at absorbing solar radiation, this is not always the case. Some butterflies near the equator have a non-melanin black colouration, which reflects rather than absorbs radiation (Heinrich 1972). In absorbence baskers, only the basal portion of the wing is relevant to absorption of solar energy to raise thoracic temperature. This is the area where the degree of melanization will be most critical for thermoregulation. In reflectance basking *Pieris* species, increased melanization in the dorsal basal area of the wing serves to increase basking body temperature, whereas increased melanization in the outer marginal area will have the opposite effect (by reducing the reflective area of the wing surface) (Kingsolver 1987a).

Variation in Melanization

Given the significance of degree of melanization for thermoregulation, one might expect to find variation in the degree of melanization along climatic gradients. Such variation would reflect the need to increase efficiency in absorption of solar energy (and hence achieve or maintain thoracic temperatures necessary for flight) in conditions of low ambient temperature and/or solar radiation.

Many species exhibit variation in degree of melanization, both within and between populations. Such variation can occur between populations in different parts of a species range (e.g. see Dennis & Shreeve 1989 and Dennis 1993 for discussion of variation in British species) or as a seasonal phenomenon, with variation in melanization between broods (seasonal polyphenism) (Shapiro 1976). Seasonal polyphenism is under environmental control, with the same genotype being able to give rise to different phenotypes, usually under the influence of temperature and/or photoperiod in the immature stages. The relationship between phenotypic and genetic variation is not necessarily clear cut, as for several species it is possible to artificially produce different "geographic" forms by manipulation of environmental variables (Shapiro 1976). The ability to produce a range of phenotypic forms may be an adaptive advantage in habitats which are subject to predictable variations in seasonal conditions (e.g. "wet" and "dry" forms of tropical *Bicyclus* species - Windig 1992).

The adaptive significance of infra-specific variation in melanization is not always apparent. Bowden (1979) reviewed data on climate, melanization and environmental determination of melanin deposition in *Pieris* spp., and concluded that geographic variation in melanization was nonadaptive, and was more explicable in terms of historical and phylogenetic relationships, rather than as an adaptation to the contemporary habitat and climate. A different approach is taken by Dennis & Shreeve (1989), who argue that variation in wing morphology in many British butterflies is correlated with contemporary environmental gradients. Shapiro (1976) also argues that geographic and seasonal variation of melanization in *Pieris* is often correlated with climate and is probably a thermoregulatory adaptation.

Several studies have investigated both seasonal and climatic (usually altitudinal) variation in melanization, and related this to an energy absorption advantage for the darker forms. Populations of *Colias* species studied in N. America show increased melanization of the ventral wings (particularly hind wings) at both higher latitudes and altitudes (i.e. cooler climates) (Watt1968, 1969). *Colias* also show seasonal variation in melanism, with darker forms being more frequent at colder times of year (Watt 1969). Darker forms have been shown to heat up faster and maintain a higher steady state temperature than lighter forms (Watt 1968), allowing them to achieve thoracic temperatures required for flight under ambient temperatures which would be limiting for lighter forms (Watt 1969). Lighter forms may be maintained in the same population as under conditions of high solar radiation they would be able to remain flying for longer when darker forms would overheat.

VanDyck *et al.* (1997a, 1997b) relate differences in degree of melanization in *Pararge aegeria* to differences in activity patterns and thermoregulation. Darker males are able to warm up more rapidly and so spend a greater proportion of the time flying in shady areas. Paler males spend more time resting in sunlit patches, where they are less likely to overheat than darker males. This also relates to mate location strategy (see below), with paler males more likely to engage in perching behaviour, whereas darker males are more likely to patrol in search of mates.

Jacobs & Watt (1994) found that seasonal variation in both melanization and "furriness" in *Colias eurytheme* were under photoperiodic control, although the two characteristics were not well correlated in their expression in individuals. They suggested that this lack of correlation might reflect either independence in the microevolutionary dynamics of the two characters, or it might signify "a real mechanistic limitation on the power of natural selection to produce "optimal" phenotypic control."

Douglas & Grula (1978) also propose an adaptive function for melanization in another lateral basker, *Natholis iole.* Degree of wing melanization is under photoperiodic control, with lighter forms being produced in the summer, when overheating is a potential problem, and darker forms in the spring and autumn. Darker forms heat up

quicker and can achieve a higher equilibrium temperature than lighter forms (37° C cf. 34° C at an ambient temperature of 24° C).

2.2.5 Additional Adaptations

Along with the morphological adaptations to the different seasonal, regional and altitudinal conditions described above, several studies also document variation in enzyme systems, which relate to flight activity. The PGI (phosphoglucose isomerase) enzyme systems are involved in producing fuel for flight through glycolysis. In *Colias,* variation in PGI allozyme frequency was found to be associated with altitude, weather and flight activity (Watt 1977, 1983, Watt *et al.* 1983, 1985). Goulson (1993) found that variation in phosphoglucose mutase (PGM) allozyme frequencies in meadow browns (*Maniola jurtina*) was associated with the ability to sustain prolonged flight. He suggests that short-term temporal heterogeneity in temperature and weather conditions could maintain the polymorphism, by favouring different genotypes under different temperature and weather conditions.

Although the current project is concerned with adaptive variation of adult butterflies, it is also important to remember that it is not just the adult imago which may be involved in local or regional adaptations. Ayres & Scriber (1994), for example, found that the key adaptation of *Papilio canadensis* to the cooler, shorter summers encountered in the northern part of its range was an increased larval growth rate.

2.2.6 Flight Space

One of the ways to evaluate whether variation between or within populations (in, for example, degree of melanization, absorptivity of wing surfaces etc.) actually translates into an adaptive advantage in the field, is to determine the butterflies' "flight space". The flight space can be defined as " the ranges of certain meteorological variables, such as solar radiation, air temperature and wind speed, in which flight can or does occur in a particular butterfly or species of butterfly" (Kingsolver 1985a).

Flight space can be evaluated empirically, and this has been done for a number of species, including *Papilio polyxenes* (Rawlins 1980) and *Pieris virginiensis*

(Cappuccino & Kareiva 1985). Roland (1982) determined the flight space for light and dark forms of the alpine butterfly *Colias nastes*, to test the hypothesis that more efficient absorption of solar radiation by dark forms would translate into prolonged daily activity. Results showed that darker individuals could heat up faster under conditions of low temperature and solar radiation, and hence could fly at lower thresholds for air temperature and solar radiation. Thoracic temperature necessary for initiating active flight (approx. 30° C) was the same for both light and dark forms. *Colias* males locate mates by actively patrolling, and spend between 50% and 90% of their time in flight, when within their flight activity range of environmental conditions (Kingsolver 1983a). Females mature and lay eggs throughout their life, and require nectar to maintain their egg output - activities, which again depend on active flight. Once flying, degree of melanization did not affect ability to carry out these activities, but the difference between dark and light forms in the amount of time which could be spent in flight (up to 55%) has significant implications for fitness in climatically marginal conditions.

Guppy (1986b) evaluated flight space for another alpine butterfly, *Parnassius phoebus,* in relation to a number of morphological characteristics, including degree of melanization. *P. phoebus*, a dorsal basker, shows variation in a number of characteristics, including melanization of the basal area of the dorsal wings. The basal patch can vary in both size and blackness (proportion of black/white scales). Unlike results for *Colias*, degree of melanization had no effect on minimum flight thresholds. However, once flying darker individuals where able to maintain active flight for longer than the lighter forms. Under conditions of low solar radiation, basal patch width was positively correlated with time spent in flight, whereas under conditions of low air temperature, patch blackness was more significant.

These two studies illustrate a number of important points about the relationship between wing morphology, flight time and weather. Both demonstrate that variation in melanization, through its effect on thermoregulation, can have adaptive significance - through its influence on flight time - under natural conditions. Differences between the two species (*Colias nastes* and *Parnassius phoebus*), in how melanization influences flight threshold and flight duration once the threshold is reached, illustrate one of the

ways in which there is an *interaction* between wing morphology (e.g. size, blackness and position of melanic patches) and behaviour (basking posture and flight style). *P. phoebus* are dorsal baskers and have a fairly slow gliding flight. This means that dorsal melanic areas, which absorb solar radiation in pre-flight warm up also, absorb radiation during flight. Thoracic temperatures during flight are maintained as the slow wing beat causes little disturbance of the boundary layer of warm air at the base of the wing. In contrast, melanization of the ventral wing surface, important for pre-flight warm up in the lateral basking, fast flying *Colias*, has no influence on thermoregulation during flight.

Another approach using the concept of flight space is to develop a model incorporating morphological, behavioural and thermoregulatory characteristics of a particular species (real or hypothetical) and physical parameters of heat gain and heat loss. This approach to modelling flight space has been developed by Kingsolver (1983a, 1983b), and Kingsolver & Watt (1983, 1984), and has a number of uses. One is to predict the range of meteorological and climatic conditions under which a particular species can or cannot fly. This has been done for *Colias philodice eryphyle* and *Colias meadii* and predictions from the model verified by the use of transplant experiments (Kingsolver & Watt 1984). Another use of this approach is to model the effect of changes in morphological characteristics, such as melanization, and predict how these changes would influence flight space.

Such approaches, incorporating morphological, behavioural, thermoregulatory and meteorological data, could prove useful for evaluating long term population dynamics of species, in the context of conservation strategies. Natural and anthropological changes in climate can have a major influence on species distribution and abundance (e.g. Dennis & Shreeve 1991). Using predictive models of flight space would contribute to an understanding of how such changes might affect a particular species distribution. As wing morphology is an important element of flight space models, the amount of variation in wing morphology (including phenotypic plasticity) present within a particular species may be one of the most important elements in determining whether that species can survive under changed environmental conditions (manifested

by either range expansion into new climatic zones, or range retention under changing climatic conditions). For example, although they did not specifically use a flight space model, Douglas & Grula (1978) argue that seasonal polyphenism - in the form of photoperiodically controlled deposition of melanin - may be one of the reasons behind the range expansion of both *Colias eurytheme* and *Nathalis iole.*

2.3 PREDATOR AVOIDANCE

Butterflies are subject to attack by a range of predators and parasitoids at each stage of their life cycle. Arthropod predation may account for high levels of mortality during the early stages of the life cycle (eggs and small larvae), whereas predation by vertebrates becomes increasingly important in the later stages - larger larvae, pupae and adults (Dempster 1984). Although adult butterflies may be eaten by other invertebrates, such as spiders, in the context of the interaction between wing morphology and predation, it is visually hunting vertebrate predators (mainly birds in Britain, but also lizards in continental Europe) which are of interest.

2.3.1 Practical Problems

Several aspects of butterfly wing morphology - e.g. the high incidence of cryptic patterns and colouration (particularly on the ventral hind wings), mimicry and aposematic colouration, all suggest that visual predation is a significant selective agent in the evolution of wing colour and pattern (reviewed by Scoble 1992). Despite this strong circumstantial evidence, direct evidence of the impact of visually hunting predators is hard to obtain in the wild. Kingsolver (1987b), in a study of predation on *Pieris* under semi-natural conditions, reports that in approximately 170 hours of observations not a single predation event was recorded where the predator could be unambiguously identified.

Not surprisingly, perhaps, Kingsolver appears to remain ambivalent about the exact role of wing colouration in predator avoidance. In earlier work (Kingsolver 1985c) it was suggested that when warming up in the morning, *Pieris* bask with their wings at a smaller angle than expected, possibly as a compromise to avoid being too apparent to predators. In the later work (Kingsolver 1987a, 1987b), there are two suggestions: one

is that white colouration in *Pieris* may be aposematic (if so, a certain degree of apparency to predators would be necessary), due to the presence of noxious and distasteful chemicals (Aplin *et al.* 1975), which makes them unpalatable to some birds (Wourms & Wassermann 1985). The other suggestion is that the butterflies' posture did not effect their predation rate, implying that visually orientated predators were not significant in this study. If this was the case, then the main influence of wing colour on predation was through its effects on thermoregulation and the butterflies' capacity to fly (Kingsolver 1987b).

Since direct predation events are hard to observe in the field, several studies have used indirect measures, such as the presence of beak marks on the wings (e.g. Bowers *et al.* 1985). The main problem with the interpretation of this sort of evidence is whether frequency of beak marks observed is an indication of overall predation rate, or rate of successful escape (Bengston 1981). In a comparison of beak mark frequency in, for example, two different morphs, a higher incidence in one could be interpreted as either: a higher predation rate on that morph (i.e. less well adapted) or a higher rate of successful escape (better adapted). This means that wing damage data cannot really be used to compare levels of predation between different populations or wing morphs (Brakefield, Shreeve & Thomas 1992).

In spite of the practical difficulties of directly observing predation in the wild, there is still a large body of work which looks at the adaptive significance of wing morphology in relation to avoiding predators. Defence mechanisms can be divided into two main categories: primary and secondary. Primary defence involves avoiding detection, whereas secondary defences are employed to try and escape from a predator once detected.

2.3.2 Primary Defence

One of the main mechanisms for primary defence (especially in palatable species) is camouflage or crypsis. The adaptive significance of cryptic colouration has been demonstrated for a wide range of organisms (see Endler 1978 for an extensive review), and can be defined as follows; "A colour pattern is cryptic if it resembles a random

sample of the background perceived by predators at the time and age, and in the microhabitat where the prey is most vulnerable to visually-hunting predators." (Endler, 1978). Butterflies are most vulnerable to predation in thermally marginal conditions, when flight is difficult (Shapiro 1974, Rawlins & Lederhouse 1978, Lederhouse 1983, Bowers *et al.* 1985, Kingsolver 1987b). When completely inactive (roosting), butterflies close their wings. This means that the ventral wing surface (especially the hind wing) has the main function of camouflaging the butterfly against its resting background. The degree to which other wing surfaces are involved in primary defence, through cryptic pattern and colours depends to a large extent on the butterfly's resting posture (dorsal or lateral) and behavioural norms, which have evolved in the context of host-plant habitat structure and climate (Shreeve & Dennis 1992). The overall wing morphology is a result of compromise between the different functions the wings must perform in relation to thermoregulation, communication and defence (Dennis & Shreeve 1988, Shreeve & Dennis 1992, Dennis 1993).

2.3.3 Secondary Defence

Once a predator has detected the butterfly, secondary defence mechanisms come into play. These include "startle" or "flash" patterns, to startle or nonfuse the predator. The large eyespots of the peacock butterfly, *Inachis io,* are good example of this, when the butterfly suddenly opens its wings when disturbed (also accompanied by a hissing sound as the fore and hind wings rub together). Other morphological modifications of the wings may serve to deflect a predator's attack away from the body and towards the outer margins of the wings, enabling the butterfly to break away without lethal damage. Such secondary defence features include eyespots, common in many Satyrine butterflies and "false heads" (modifications of the hind wings, including "tail" extensions which are supposed to resemble antennae) which are found in many Lycaenids and some Papilionids (Scoble 1992). The probable function of these structures in secondary defence has been demonstrated by Wourms & Wasserman (1985), who manipulated the wing morphology of *Pieris* butterflies to test whether false heads and eye spots were an effective defence adaptation. They found that the presence of false heads led to a significant increase in attacks being redirected to the hind wing, and that they increased the probability of escape once attacked.

A number of species show variation in the number, size and apparency of eye spots both within populations (e.g. Shreeve 1987) and between populations in different parts of their range (Dennis & Shreeve 1989). Several studies have attempted to relate variation in spot pattern and number to differences in levels of activity (often climatically mediated), and hence exposure to predators (e.g. Brakefield 1979, Bengston 1981, Dennis, Porter & Williams 1986). So one explanation for variation in spot pattern between populations is that differences between activity rhythms may shift the balance between the need for primary defence (crypsis) and secondary defence (e.g. deflecting devices) (Brakefield, Shreeve & Thomas 1992).

2.3.4 Chemical defence

Adult butterflies, which contain noxious or distasteful chemicals, are often aposematic - displaying bright, contrasting colours as a warning to predators (Boppré 1984). Even though warning colouration relies on advertisement for its effectiveness, there may still be advantages in being cryptic in the first instance. This means that it is not unusual for aposematic patterns and colours to be highly apparent at close range, but still be cryptic at a distance (Endler 1978). Mimetic resemblance is often associated with this phenomenon, both Batesian (palatable species mimicking unpalatable) and Mullerian (two or more chemically protected species displaying the same warning colours) (Turner 1977, 1984). Although there has not been much work done on chemical defence and mimicry in British butterflies, it has been suggested that the white *Pieris* species may represent a loose form of Mullerian mimicry, and the white and black patterned marbled white (a Satyrine) may be a Batesian mimic of the *Pieris* species (Brakefield, Shreeve & Thomas 1992).

2.4 MATE LOCATION AND COMMUNICATION

A further function of the colouration and pattern of butterfly wings, along with thermoregulation and predator avoidance, is for intra-specific communication, such as social signals used during courtship. Darwin (1880) suggested that the sexual dimorphism, with bright male colours, found in many species of butterflies could be explained by sexual selection and female choice. More recent work, on both butterfly

mate location behaviour and visual systems, has suggested that although female choice is probably not the driving force behind the evolution of bright male colours, aspects of wing morphology (such as colour and overall pattern) can play an important role in mate acquisition behaviour.

2.4.1 Butterfly Vision

The intra-specific communicative function of butterfly wing morphology can only be understood in the context of the butterfly visual system. Butterflies have the broadest visible spectrum known in the animal kingdom, extending from red to ultra-violet. Swihart (1967) looked at spectral efficiency curves for six species of different coloured butterflies and found that within the broad visual spectrum, each species was most sensitive to the colours approximating to their own wing pigmentation. Butterflies have a wide field of vision, but low spatial resolution, which means that they are probably unable to distinguish fine pattern details unless at very close range. They also have high temporal resolution and are good at detecting moving objects, especially if the colour and brightness differ from that of the background (Silberglied 1984). An understanding of the butterfly visual system suggests that fine pattern details of wing morphology are unlikely to play a role in intra-specific communication at anything other than close range, whereas colour (including ultra-violet) and movement may have potential for detection and identification of con-specifics.

Although fine pattern detail may only come into play at close range, overall colour and contrast have been shown to play an important role in some species. Wiernasz & Kingsolver (1992), for example, demonstrated that females of the morphologically similar, sympatric species *Pieris occidentalis* and *Pieris protodice* used degree of melanization on the dorsal forewings to discriminate between hetero- and conspecific males.

2.4.2 Mate location behaviour

In some species of butterflies many females mate only once, whereas males are capable of mating a number of times. Once mated, females of such species usually avoid or reject males (e.g. Wiklund 1982), so males usually have to compete for the relatively

scarce resource of virgin or receptive females. Even in species where females mate a number of times, individual females usually have an unreceptive period of 3 - 5 days between matings, so competition between males to gain access to receptive females may still be intense. Scott (1974) identified two main methods by which males locate females; patrolling or perching. In the former, males fly in search of settled or flying females. In the latter, settled males fly up to intercept passing females, who may visit known perching areas in search of mates (Wickman 1986). Although perching and patrolling are still recognised as major mate locating strategies, other methods of mate locating behaviour are also acknowledged. These include hilltopping (males move to specific topographic locations to find mates (Shields 1967) and assemblies (leks) where males aggregate at specific sites and wait for mates (although these strategies could also be considered as part of the perch-patrol continuum - Dennis & Shreeve 1988).

Scott (1974) saw mate-locating behaviour as fairly inflexible, and best understood in a phylogenetic context. In contrast, Ehrlich (1984) emphasised the flexibility of mate-locating behaviour and the importance of current ecological circumstances. Dennis & Shreeve (1988) argue that the evolution of mate-locating behaviour can be understood in the context of the species' ancestral habitat. Patrolling evolves in a habitat structure where host-plants are either widespread and relatively uniform (e.g. grassland) or where there are no distinctive topographical features. Where host-plants are patchy, with distinctive topographical features, then perching may evolve. Many, but not all, patrolling species are brightly coloured and apparent - aiding location at a distance.

Rutowski (1991) reviews the diversity of male mate-location tactics. He argues that an appropriate framework for analysis is to look at the ecological correlates and constraints associated with *where* mate encounter sites occur (e.g. resource-based sites such as larval food plants, or non-resource-based sites such as hilltops or landmark features) and *what* the males do when they are there (e.g. perch, fly, defence). This approach recognises the diversity and flexibility of mate locating tactics (the "perch-patrol continuum") but suggests that behaviour at encounter sites (e.g. time of day visited, site tenacity and defence, perching or flying) should be considered as separate variables, as they may evolve independently.

Using the "where" and "what" approach, Rutowski *et al.* (1994) give an example of thermal constraints on male butterflies at mate encounter sites. The male desert hackberry butterfly (*Asterocampa leila*) perches on or adjacent to larval food plants, waiting for newly eclosed (emerged from pupa) and receptive females. At these sites the male posture, orientation and perch preference changes with air temperature, so that body temperature remains largely independent of air temperature. Rutowski *et al.* argue that thermal requirements, which may lead to abandoning perch sites and moving into shady areas with poorer visibility at high temperatures, may constrain the ability of males to detect mates during the hotter parts of the day.

Although some species are exclusive patrollers (e.g. *Anthocharis cardamines, Leptidea sinapis*), much recent work has demonstrated that there is a continuum of behaviour between the extremes of perching and patrolling and seeks to clarify the constraints and benefits which may lead to the adoption of a particular mate-locating strategy. Variation in mate locating behaviour can be found between different individuals in the same population. In *Pararge aegeria*, for example, males with three hind wing spots tend to patrol in open habitats, whereas those with four defend sunspots in dense woodland (Shreeve 1987). There may also be changes in the behaviour of individual butterflies depending on time of day and weather, which will influence the cost/benefit of a particular mate-locating strategy. Van Dyck *et al.* (1997a, 1997b) also studied the influence of wing colour and relative body length on male behavioural strategies in *P. aegeria.* They found that perching males were paler, with shorter bodies than patrollers. They related differences in activity between pale males (which spent most of their time resting in sunny patches) and darker males (which spent more time flying in shady areas) to differences in thermoregulation.

Males of the small heath butterfly, *Coenonympha pamphilis*, have been shown to switch between perching and patrolling strategies during the course of the day. In the morning, when air temperatures are lower, males perch and fly up to intercept passing females. As air temperatures rise later in the day, males switch to patrolling (Wickman 1985). The change in behaviour is probably associated with cost of flight and probability of

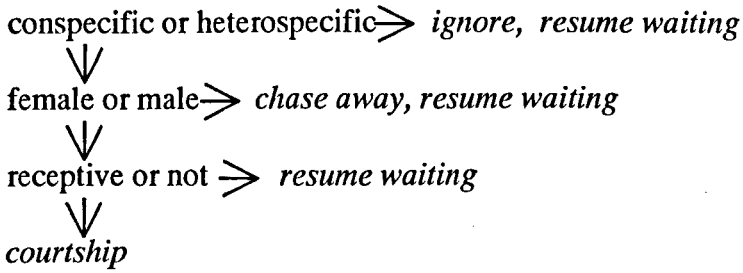
encountering virgin females. As temperatures increase, flight can be sustained for longer periods, which may help increase the chances of finding an unmated female. The hilltopping butterfly *Lasiommata megera* also shows a switch between stationary and vagrant behaviour; the latter being associated with higher temperatures and levels of solar radiation (Wickman 1987). Temperature is not the only factor affecting switches in mate location behaviour; the large skipper, *Ochlodes venata* has an opposite pattern to that of *C. pamphilus*, switching from patrolling in the morning to perching in the afternoon (Dennis & Williams 1987). The important factor here seems to be female receptivity, with females being more receptive in the morning. By afternoon, a high proportion of females will already be mated, so patrolling becomes uneconomical.

Availability of receptive females appeared to be the main factor influencing male activity in the desert hackberry butterfly, *Astercampa leilia* (Rutowski *at al.* 1996). Males were found to be most active in the morning, when females usually eclosed, at around 8.00 am, over a wide range of temperatures.

Individuals of the California patch butterfly (*Chlosyne californica*) also show the behavioural flexibility to switch between hilltop territoriality and downslope patrolling (Alcock 1994). Patrolling was associated with higher air temperatures and increased density of foraging females (some of which would be sexually receptive).

Within each major mate locating strategy (e.g. perching or patrolling) there is also a range of behaviours, which varies between species. Patrolling species may either range over a large area in search of receptive females (e.g. *Pieris napi* and *P. rapae)*, seldom returning to the same area; or may use a smaller area and repeatedly investigate the same locality (e.g. *Leptidea sinapis, Anthocharis cardamines*). Species which use perching as a mate locating strategy also show a range of behaviours. In some species there appears to be no territorial defence of perch sites (e.g. *Maniola jurtina, Cupido minimus*). In others there may be ritual displays fights in defence of territories (e.g. *Inachis io*), or real physical "collision" fights (e.g. *Pararge aegeria, Ochlodes venata*) (reviewed in Shreeve 1992). Most perching males will fly to inspect passing insects. The duration of these encounters appears to depend largely on what the males find.

Ravenscroft (1994) found that the male chequered skippers (*Carterocephalus palaemon*) were territorial within a small area. Perch sites chosen did not represent resources for females (such as nectar or larval food plants) but were apparently chosen for favourable temperature and good visibility. From these perch sites males would make short but frequent flights to intercept other insects. The duration of these interception flights depended on the nature of the other insect. Heterospecific encounters were typically brief (mean 2.8 s) with males returning to the perch after a brief inspection. Conspecific male-male encounters (chasing male from the area) were longer (mean 9.1 s) and male-female encounters longest (mean 12 s). The length of encounter presumably reflects the information the male has to acquire and hence the appropriate behaviour to adopt for each encounter:



The male white peacock butterfly (*Anartia jatrophae*) also shows territorial defence of perch sites (Lederhouse *et al.* 1992). In this species defended sites always contained a larval host plant. As in *C. palaemon,* encounters with conspecific males (mean 38 s) lasted three times longer than those with other insects. Dreisig (1995) found the same pattern in territorial male grayling (*Hipparchia semele*) and large skippers (*O. venata*), with conspecific male-male encounters lasting 3 - 4 times longer than those with other insects. Males also made "spontaneous" flights from the perch site, the frequency, but not the duration, of which increased with temperature. Dreisig suggests that these flights were not directly involved with either "inspection" or territorial defence, but functioned as a sexual signal, with the male advertising its presence to females. The cost associated with these flights would be increased conspicuousness to predators, with the associated risks.

2.4.3 Signals used in mate acquisition

The importance and nature of visual signals (movement, size, shape, colour and pattern) in mate acquisition appears to differ both between species and sexes. In all mate locating strategies, it is the males which are usually the more proactive, competitive sex. Males initially locate females using fairly unspecific signals of colour (including ultraviolet in some species) size and movement (Silberglied 1984). Choosing a perch with good visibility may be one of the factors influencing choice of site (Ravenscroft 1994). Broad pattern detail (degree of melanization) and possibly conspicuousness (contrast against the background) has been demonstrated to influence mate choice by males of the closely related species *Pieris occidentalis* and *P. protodice* (Wiernasz 1995).

Silberglied (1984) argues that evolution of bright male colouration in many species may also be influenced by male (rather than female) discrimination on the basis of colour - i.e. the need to recognise and be recognised by other males. Males, whether perching or patrolling, will often fly to investigate any object which approximates to a conspecific female. If the object turns out to be another male, then it is obviously advantageous to be able to discriminate and recognise "maleness" at the earliest opportunity. Similarly, it would pay to advertise "maleness" from a distance, to avoid wasting time with an inappropriate courtship. Silberglied (1984) used this argument to suggest that male-male interactions and long distance communication is likely to be a strong selective force leading to bright male colouration.

In many, but not all species, olfactory rather than visual signals seem to be most important for females, although the importance of female choice on the basis of visual signals has been demonstrated for several species. Female *Colias eurytheme,* for example, can distinguish between conspecific males and those of closely related *C. philodice* on the basis of UV absorption properties of the wings (Silberglied & Taylor 1978). Female *Pieris occidentalis* have been shown to use pattern and degree of melanization to discriminate between hetero- and conspecific males (Wiernasz & Kingsolver 1992).

The papers by Wiernasz & Kingsolver (1992) and Wiernasz (1995) demonstrate that degree of melanization influences not only recognition of conspecifics, but also intraspecific mate preference in both male and female *Pieris occidentalis*. Individuals of the closely related species *P. protodice* (lighter) and *P. occidentalis* (darker) represent a continuum in degree of dorsal forewing melanization. Female *P occidentalis* show a preference for more heavily melanized males, which not only ensures rejection of the lighter *P. protodice* males, but also leads to a preference for more heavily melanized males of their own species. In contrast, male *P. protodice* show a significant preference for *less heavily* melanized females of their own species. Wiernasz (1995) argues that males may benefit by discriminating against darker females. Increased basal wing melanization allows shorter flight initiation time under low temperature conditions, but may also lead to overheating when its hotter. At the low altitude site where this study was conducted, the risk of overheating would probably outweigh the benefit of decreased flight initiation time for heavily melanized females. This would mean that darker females would have a shorter lifespan and decreased reproductive success. Wiernasz also suggests that female apparency (lighter females being more visible to males) plays a part in mate choice. This opens the question of whether there is also a cost to lighter females in being more apparent to predators.

These studies on male and female mate preference, on the basis of wing melanization, open several interesting questions about the interaction of the different functions of wing morphology (in this case thermoregulation, mate acquisition and possibly predation) and the influence of different selective pressures associated with both male and female reproductive biology and climatic variables. For males, females are a limited resource, so potential lifetime reproductive success of a female (which may be influenced by wing morphology) should play an important part in mate choice. For females, males are seldom in scarce supply, so using cues which are the most reliable in discriminating between species (heavier melanization) may outweigh the cost of "coincidentally" preferring males which may overheat and have reduced life spans. One could also ask whether at higher altitudes, where cooler temperatures should favour the more heavily melanized females, male preference alters accordingly.

Questions arising from the work discussed above provide an example of where an "integrated" approach to the study of butterfly behaviour, distribution and wing morphology is of value.

2.5 AN INTEGRATED APPROACH TO BUTTERFLY WING MORPHOLOGY

Many studies, including a number of those cited above, have addressed questions involving correlations between aspects of wing morphology (e.g. degree of wing melanization) and behavioural/physiological mechanisms (e.g. thermoregulation) in the context of environmental conditions (e.g. latitudinal/elevational patterns). The role of wings in thermoregulation has also been incorporated into a few studies which are looking primarily at other aspects of behaviour (e.g. the work of Wiernasz & Kingsolver 1992) and Wiernasz (1995) on mate location strategy, mate preference, melanization and thermoregulation cited in previous section). Wickman (1992) argues that mate locating strategy may have a predictable relationship with wing morphology and thermoregulation. In perching species, basking can take place "passively" while waiting at the perch site, and selection should favour a wing design, which facilitates rapid take-off, approach and manoeuvrability. In contrast, patrollers should be selected for increased flight endurance and flight time. This may involve decreased relative flight costs (e.g. larger relative wing area) or a decrease in time needed for basking and thermoregulation.

Srygley & Chai (1990), Chai & Srygley (1990) and Srygley (1994) have investigated the relationship between palatability, wing morphology, behaviour and thermoregulation in a number of tropical species of butterflies. They found that palatable species tended to have a fast, erratic flight compared to the slower more regular flight of unpalatable butterflies (which might increase the effect of advertising aposematic colouration). The flight style of palatable species is more energetically expensive and demanding, which was reflected in their microhabitat choice and pattern of daily activity. Palatable species were found to fly with a higher thoracic temperature than unpalatable, and generally flew at warmer times of day and in microhabitats with a higher ambient temperature.

In the last few years Dennis and Shreeve have produced a series of theoretical/review papers which take an explicitly integrative approach to exploring functional aspects of wing morphology and morphological variation, in relation to behaviour, climate and habitat structure (Dennis & Shreeve 1988, 1989, Shreeve & Dennis 1992). They argue that the allocation of different functions to each wing surface is dependent on the posture adopted by the resting, but potentially active butterfly (e.g. wings open or closed), which is conservative within taxonomic groups.

Selection on different aspects of wing morphology associated with these functions (e.g. brightness and apparency for mate acquisition, cryptic patterns and deflecting patterns for predator avoidance and degree of melanization for thermoregulation) will be associated with constraints imposed by climate and habitat types, which may influence activity patterns and chance of encountering mates or avoiding predators. The resultant wing morphology can be described as a compromise between the requirements for mate attraction, predator avoidance and thermoregulation.

Dennis & Shreeve (1989) explore the nature of morphological variation in the British Isles, in relation to climate, behavioural posture and habitat structure. They argue that much of this variation can be understood in terms of the need to maintain activity in thermally marginal environments (e.g. increased melanization for higher absorptivity of solar radiation). There may also be shifts between the requirements of primary or secondary defence, related to activity patterns, along with variation in brightness and apparency related to changing circumstances for locating mates (e.g. time available for flight, population density). In species which rest with their wings open when potentially active, the dorsal wing surfaces may function for thermoregulation, conspecific signalling, and secondary defence. Changes on the dorsal wing surface, such as increased apparency to facilitate mate acquisition, will not compromise the primary defence (cryptic) pattern of the ventral wing surface. In contrast, the ventral wing surface of lateral baskers, which is exposed at all times when the butterfly is not flying, must fulfil all the functions of thermoregulation, communication, primary and secondary defence. In this case, constraints on the partitioning of functions between

different wing surfaces may lead to potential conflicts between, for example, the need for crypsis and apparency.

The ability to maintain activity levels which are compatible with minimum requirements for mate acquisition, feeding, egg production and predator avoidance may be a critical factor delimiting a species range. Adjustment of wing morphology is one of the key factors which may enable a species to exist in a range of climatic zones, and may be one of the factors determining the climatic limits to a species distribution (e.g. Douglas & Grula 1978). Modification of wing morphology in response to the selective pressures associated with thermally marginal environments, indicates that the species has sufficient genetic variability and/or phenotypic plasticity to allow expression of the morphological variation. If species lack the genetic variability or phenotypic plasticity which permits variation in wing morphology, this may impose constraints on the range of geographic and climatic conditions in which it can survive.

2.6 The Meadow Brown

Maniola jurtina is found throughout much of the western Palaearctic region, from the Canary Islands and North Africa in the south west, to Scandinavia in the north and to the Urals, Asia Minor and Iran in the east. In the U.K. its range extends to the far north of Scotland , although it is absent from most northern upland and intensively farmed areas (Emmet & Heath 1989). It is found on a range of grassland sites, with highest densities occurring on unimproved grassland of intermediate height, with abundant flowering plants such as knapweed. Data from the Butterfly Monitoring Scheme, for 1976 - 1992, shows that *M. jurtina* has increased in abundance in recent years (Pollard *et al.* 1995).

Geographic and seasonal variation in wing morphology are both apparent, with four geographic variants being recognised in the UK (type localities given in brackets): *insularis* (England; Isle of Wight), *iernes* (Ireland; Co. Kerry), *cassiteridum* (England; Isles of Scilly) and *splendida* (Scotland; Longa Island). Emmet & Heath (1989) suggest that these sub species may represent extremes of a continuous morphological variation, rather than discrete forms. Seasonal variation shows a trend for both size and number of

hindwing spots to decrease throughout the flight season (Brakefield 1984, Emmet & Heath 1989, Brakefield & Shreeve 1992).

Females mate only once, usually shortly after eclosion. Eggs mature in the abdomen after emergence and are laid singly , at the base of grass blades or on nearby dead plant material (Wiklund 1984). *M. jurtina* is a univoltine species, which overwinters as a small larva, feeding on fine-leafed grasses. The flight period extends from late June to September or October in the south, with an extended period being characteristic of short turfed chalk grassland sites (Emmet & Heath 1989, Shreeve 1989). In the north of Scotland the flight season is much shorter, extending only from late June to early or mid August, with lower population densities (Brakefield 1982b). Much of the variation in the phenology of meadow brown populations can be explained by differences in June temperatures (Brakefield 1987). As in many butterflies species *M. jurtina* exhibits protandry, with males emerging up to two weeks earlier than females (Emmet & Heath 1989, Shreeve 1989).

Meadow browns have been described as a fairly sedentary species with a small home range of approximately 0.85 ha (although this is probably an underestimate), and with limited long distance movement (Ford 1975, Pollard 1981, Brakefield 1982a). Some authors, however, suggest that long distance movements are not uncommon and that the meadow brown is a highly mobile species (Shreeve *et al* 1996). Life expectancy for males and females is 5 - 12 days (Brakefield 1982b).

CHAPTER 3. MATERIALS AND METHODS

A combination of field observations and laboratory methods were used in this study. A general account of the materials and methods used in collecting data from two geographic areas, over three years, is given here. Further details of specific techniques and manipulation of the raw data is given in subsequent chapters, where relevant.

3.1 Field work study sites

Field work took place in two geographic areas; southern-central England (subsequently referred to as southern population) and NW Scotland (subsequently referred to as northern population). The sites were chosen to represent a range of habitats in which meadow browns are found and (with the exception of the woodland site in southern England) to provide corresponding habitat structures in the English and Scottish study areas.

3.1.1 South Central England

Observations were carried out at three main sites on the Oxfordshire-Buckinghamshire border and in the Chilterns; Bernwood Meadow, Bernwood Forest and Grangelands.

Bernwood Meadow (SP606110)

Bernwood Meadow (Figure 1) is an SSSI, owned by the Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust (BBONT), on the Oxfordshire-Buckinghamshire border. The site is on level ground at an altitude of approximately 70 m. The main meadow consists of herb-rich neutral grassland, bordered by a path and hedges. The meadow is cut for hay in July and grazed by cattle later in the season. Potential nectar plants for the butterflies include; meadow buttercup (*Ranunculus acris*), field scabius (*Knautia arvensis*), thistles (*Cirsium spp.*), bird's foot trefoil (*Lotus corniculatus*), knapweed (*Centauria nigra*), red clover (*Trifolium pratense*) and ox-eye daisy (*Chrysanthemum leucanthemum*). Grass species include; Yorkshire-fog (*Holcus lanatus*), meadow fescue (*Festuca pratensis*), meadow-grass (*Poa spp.*), cock's-foot (*Dactylis glomerata*), false oat-grass (*Arrhenatherum elatius*), and Timothy (*Phleum pratense*).

Figure 1. Bernwood Meadow

The most abundant butterfly species encountered in the meadow (other than the meadow brown) included the gatekeeper (*Pyronia tithonus*), frequently seen in the hedges surrounding the meadow, and common blues (*Polyommatus icarus*). The marbled white (*Melanargia galathea*) was also frequently encountered flying over the long grass, and ringlets (*Aphantopus hyperantus*) were found near a ditch at one end.

Bernwood Forest (SP611102)

Bernwood Forest (Figure 2) lies approximately 10 km south west of Oxford, adjacent to Bernwood meadows. The rides used as study sites are on level ground, at an altitude of approximately 70 m. Although much of the forest is managed commercially (by Forest Enterprises), the rides and intersections have great nature conservation value and are managed jointly with English Nature (with a special interest in their potential for the pearl-bordered fritillary, *Boloria euphrosyne*). Although ancient, most of the forest is now planted with conifers (mainly Norway spruce (*Picea abies*), but also some larch (*Larix decidua*), Western hemlock (*Tsuga heterophylla*) and Scots pine (*Pinus sylvestris*). Many of the rides are lined with deciduous trees and shrubs, including oak (*Quercus robur*), hawthorn (*Crataegus monogyna*), birch (*Betula pendula*) and ash (*Fraxinus excelsior*).

Most of the observations in Bernwood Forest took place in an open, grassy ride in the south west section of the forest and at a nearby grassy glade intersection. Common grasses along the rides include bent (*Agrostis* spp.), *Poa* spp., tufted hair-grass (*Deschampsia cespitosa*) yorkshire fog, fescue and meadow grass. Flower species along the rides and in the grassy glade include the common spotted orchid (*Dactylorhiza fuchsii*), creeping buttercup (*Ranunculus repens*), blackberry (*Rubus fruticosus*), wood avens (*Geum urbanum*), bugle (*Ajuga reptans*), vetches (*Vicia* spp.) thistles and ox-eye daisy.

Apart from meadow browns, other butterfly species most frequently encountered were the ringlet, gatekeeper and in the shadier parts of the ride, the speckled wood (*Pararge aegeria*). In the grassy glade the marbled white was also frequent.

Figure 2. Bernwood Forest: ride (top) and grassy glade intersection (bottom)

Grangelands (SP829045)

Grangelands (Figure 3) is an SSSI on the western edge of the Chilterns, NE of Princes Risborough. It is a chalk grassland site, managed by BBONT, with intermittent sheep grazing. The area used in this study lies on a south facing slope at an altitude of approximately 200 - 230 m. The site features two parallel ridges, with dividing valleys, providing a variety of aspects and habitats. The ridges are dominated by sheep's fescue (*Festuca ovina*), with a rich herbaceous flora. Common flower species include; bird's foot trefoil, dwarf thistle (*Cirsium acaule*), thyme (*Thymus praecox*), hawkweed (*Hierachium pilosella*), horseshoe vetch (*Hippocrepis comosa*) and small scabius (*Scabiosa columbaria).* Patches of scrub throughout the site include species such as dogwood (*Cornus sanguinea*), privet (*Ligustrum vulgare*), dog rose (*Rosa canina*), buckthorn (*Rhamnus catharticus*) and hawthorn (*Crataegus monogyna*).

In the valley between the ridges, coarser grasses, scrub and taller herbs are more frequent. One area includes a large patch of marjoram (*Origanum vulgare*), a favourite feeding area for the butterflies and day flying silver-Y moths (*Autographa gamma*). Knapweed and scabius are also abundant.

The site has a rich butterfly fauna, with abundant species including the common blue, chalkhill blue (*Lysandra coridon*), brimstone (*Gonepterix rhamni*), large skipper (*Ochlodes venata*) and small heath (*Coenonympha pamphilus*). A clouded yellow (*Colius croceus*) was seen on two occasions.

3.1.2 NW Scotland

Observations took place at two sites on the Ardtornish Estate, on the Morvern peninsula in Argyll.

Gleann Geal (NM735504)

This site, known as The White Glen (Figure 4), consists of an area of south facing slope on each side of the A884. Beinn Chlaonleud (478 m), rises steeply at the northern edge of the site, and a shallow river forms the southern boundary. A small oak woodland (*Quercus petraea*) forms the western boundary of the site and scattered oaks occur along

Figure 3. Grangelands

Figure 4. Gleann Geal

the river bank. The altitude over which observations took place is between 50 m and 100m. To the south of the road, the land is predominantly short turf, dominated by *F.ovina*, with numerous rocky outcrops. Other grass species include *Agrostis* spp. and *H. lanatus*. The dominant nectar plant is thyme, which occurs in scattered clumps, particularly on a steep bank just below the road.

To the north of the road is a flatter, wetter area at the base of the hill. This area is dominated by mat-grass (*Nardus stricta*) and purple moor-grass (*Molinia caerulea*), with patches of sphagnum, cotton-grass (*Eriophorum angustifolium*) and *Juncus* spp. On the lower slope of Beinn Chlaonleud the vegetation is dominated by bracken (*Pteridium aquilinum*) with large areas of bog myrtle (*Myrica gale*). Flowering herbaceous plants include butterwort (*Pinguicula vulgaris*), violet (*Viola* spp.) and bog asphodel (*Narthecium ossifragum*).

The most frequently encountered butterfly, after meadow browns, was the Scotch argus (*Erebia aethiops*).

Kinlochaline field (NM 695478)

Kinlochaline field (Figure 5) lies on a south facing slope at an altitude of approximately 50 m, to the north of Loch Aline. It is an area of herb rich rough grassland, parts of which are cut for hay in July. The field is then grazed by cattle later in the season. Grass species include *F. ovina, P. pratense, Agrostis* spp. and *H. lanatus*. The field contained large patches of nettles (*Urtica dioica*) where small tortoiseshell butterflies (*Aglais urticae*) were seen emerging from their pupae. Thistles are also abundant and other herbaceous species include; buttercups, knapweed, hawkweed, bugle, red and white clover (*Trifolium repens*), harebell (*Campanula rotundifolia*), ragged robin (*Lychnis flos-cuculi*) and ox-eye daisy.

Small tortoiseshells were abundant at this site and the dark green fritillary (*Argynnis aglaja*) was a frequent visitor.

Figure 5. Kinlochaline Field

3.2 Behaviour and Environmental Variables

An important part of this study involved recording environmental variables which were thought to influence butterfly behaviour. To make these data of optimum value, environmental variables were recorded at the sites where behavioural observations took place (rather than using regional weather data). It was also important to try and ensure that the time scale of recording environmental variables was appropriate to the time scale at which changes in butterfly behaviour took place - but without making the data so fine-scale as to be unmanageable. An interval of one minute was chosen as an appropriate level of detail. Observations of butterfly behaviour were recorded and timed as accurately as possible, to the nearest few seconds. Data on butterfly behaviour could then be combined with environmental data for the one minute interval during which the behaviour occurred.

3.2.1 Environmental Variables

At all times when behavioural observations were taking place, recordings were made for the following environmental variables: ambient air temperature (T_a), air temperature at the height at which most butterfly flight occurred (T2), ground temperature (T3), wind speed and solar radiation (SR).

Environmental variables were recorded using a seven channel data logger (Skye Instruments : SDL 2960), mounted on a tripod, approximately 80 cm above the ground. The data logger was set up in an open area, close to where the behavioural observations were made. The probes connected to the data logger were: an anemometer (Skye Instruments : A100R/I) mounted on a pole at a height of approximately 1.8 m (wind speed, m min^{-1}), pyranometer (Skye Instruments : SKS 1110/I), mounted on a levelling unit, height 130 cm (solar radiation, Wm^{-2}), integral shaded thermistor (T_a) (ambient temperature, °C), two sheathed thermistor probes (Skye Instruments: SKTS 200) to record temperatures at ground level (T3) and in the vegetation (T2) at a height of 50 - 100 cm where butterflies tended to be most active; flying, feeding, etc. (Figure 6). The data logger was set to take the full set of readings for every minute during the periods of behavioural observations. These were then downloaded onto an EXCEL spreadsheet to enable behavioural data to be matched to environmental variables.

Figure 6. Data logger set up during field work

A = anemometer
B = pyranometer
C = data logger with integral shaded thermistor

3.2.3 Behavioural observations

Behavioural observations in the field were carried out on the southern population between 22 June and 7 September 1994. The Scottish population had a much shorter flight season and observations took place between 12 July and 31 July 1995. All observations in both regions took place between 08.00 and 19.00. Many of the data sets for the Scottish population are smaller than those for the southern population, due to the shorter flight season limiting time available for observations, and the lower density of butterflies in the northern population.

Behavioural observations consisted of a combination of transects and detailed observations of individual butterflies. Using these two methods of recording was necessary as each has associated advantages, biases and limitations, but together they provide different, complementary, types of data. Transects, for example, have the advantage of collecting data for the general population levels of activity - but with an obvious bias towards recording the more "visible" categories of activity, such as flight. Following individual butterflies gives more detailed information about all behavioural categories, including duration of particular activities and environmental variables associated with transitions between activities (e.g. flight initiation). A potential bias of this type of data is that it is prone to individual bias, i.e. the individual under observation may not be a typical representative of the population. It is also possible that pooled data from all the individuals tracked may represent a particular subset of the total population; e.g. if only the more "obvious" individuals, or those which tend to stay in a limited area, are accessible to prolonged observation. For transects, each data point can be treated as independent, as each represents contact with a separate individual. For "following" , for many of the data sets, the appropriate level of analysis is to use summary values from each individual (e.g. mean, minimum and maximum ambient temp over which flight occurred) as the data points from a single individual cannot be treated as independent in the same way as for transect data. Once summary data for each individual have been extracted, these can then be pooled for species, sex etc. in the same way as for transect data. This allows detailed population data to be obtained for all behaviour categories, including those such as "alighted" which are likely to be under-recorded in transects. Another advantage of following individuals is that information

can be obtained on the sequence and duration of each behaviour, under different environmental conditions.

3.2.3.1 Transects

Transects were conducted by walking a set route, at a steady pace, within each study site. Individuals were recorded if they were within a 5 m "box" of the position on the transect route (following the system adopted by the Butterfly Recording Scheme, Pollard & Yates (1993). For each individual encountered recordings were made of the time of observation (to the nearest 5 seconds, using a digital watch synchronised with the internal data logger clock), sex and behaviour (see below, section 3.2.3.2, for description of behavioural categories). An additional category of "flew when disturbed" (FWD) was included for behaviour recorded during transects. This behaviour consisted of a settled butterfly flying when disturbed by the recorder walking past, and landing again within a few metres. Recordings were made using a hand-held dictaphone. Tapes were later transcribed and data entered into an EXCEL spreadsheet and the times associated with each activity in the field synchronised with the timed environmental data, which had been recorded simultaneously by the data logger.

A total of 1067 transect-minutes and 1193 butterfly contacts, with associated environmental variables, were recorded for the southern population. A total of 591 transect-minutes and 93 butterfly contacts, with associated environmental variables, were recorded for the northern population.

3.2.3.2 Following individuals

Following individuals (using protocols based on those developed by Shreeve 1984, 1985) entailed following a butterfly until contact was lost, and recording all activities.

Activities were divided into the following categories:

alighted (including posture and orientation)
flying
chasing
feeding
courting/mating
walking
egg laying

A hand held compass was used to establish orientation of alighted butterflies. Wing angle, recorded as estimated angle between the wings (e.g. -5° = wings fully open and oppressed against the substrate, 180° = wings fully open, 90° = wings held at right angles to each other, 0° = wings closed) was estimated to within +/- 5 - 10° with the help of a hand held protractor, lined up with the butterfly. All activities were timed to the nearest 5 seconds (digital watch synchronised with the internal data logger clock). Height (estimated using 1 m rule), position and distance travelled (estimated by recording the number of paces between butterfly landings and later converting this to metres) were also noted. As before, recordings were made using a hand-held dictaphone. Tapes were later transcribed and data entered into an EXCEL spreadsheet and the times associated with each activity in the field synchronised with the timed environmental data, which had been recorded simultaneously by the data logger.

For the southern population a total of 96 individuals were followed for 1144 minutes. Figures for the northern population were 57 individuals for 1167 minutes.

3.3 Thoracic temperatures in the field

Most butterflies need to achieve thoracic temperatures (T_{th}) of approximately 30°C before flight is initiated (Kingsolver 1985a). In this project we wanted to determine the thoracic temperatures of active meadow browns, how thoracic temperature was affected by ambient temperature and solar radiation, and whether thoracic temperatures required for flight initiation differed for northern and southern populations.

Thoracic temperatures of butterflies in the field were obtained during the course of field work in 1994 and 1995. Additional data were collected for the southern population in July 1996.

Temperatures of butterflies in the field were obtained by using the "grab and stab" technique, a common method of obtaining thoracic temperatures of butterflies in the field (e.g. Shreeve 1984, Shelly & Ludwig 1985, Guppy 1986b, Pivnick & McNeil, 1987). A 0.4 mm diameter thermistor probe was embedded in the tip of a hypodermic needle and connected to a hand held digital multimeter. Readings of resistance of the thermistor were taken directly from the multimeter, and later converted to temperature. Butterflies were caught in a net and the hypodermic needle inserted through the net into the dorsal thorax. This enabled a measure of thoracic temperature to be recorded within approximately 15 seconds of capture.

Time of capture and activity at the time of capture were recorded. Simultaneous data were collected for the environmental variables of solar radiation, ambient air temperature, temperature at vegetation height, ground temperature, and wind speed

3.4 Laboratory Analysis

To complement data collected from observations of butterflies in the field, laboratory methods were used to obtain more detailed information on the rates at which butterflies warmed-up under artificial light, how this was influenced by wing morphology and whether there was any difference between the northern and southern populations.

Laboratory methods were used to quantify various aspects of wing morphology. Data on wing morphology were used to investigate whether there were any differences between the northern and southern populations, which might be expected from the effect of wing morphology on thermoregulation (Dennis 1993).

Butterflies used for these experiments were caught in July 1996, at Bernwood Meadow (southern population) and at Ardtornish and the surrounding area (northern population).

Butterflies were kept alive before the experiments (for a maximum of 10 days) by placing them in individual pill boxes in a domestic refrigerator. Every 2 days the butterflies were brought into room temperature conditions and allowed to feed on dilute honey water. Butterflies from the northern population were transported south in paper envelopes, placed in a cool box. On arrival they were placed in small flight cage and allowed to feed on fresh cut flowers and honey water, before being transferred to individual pill boxes. Having live specimens from both northern and southern populations allowed simultaneous testing of individuals from the two populations. The wings of each individual were then retained for digital image analysis of wing morphology. For butterflies caught in Bernwood Meadow, information was also obtained for time of day and values for environmental variables (see section 3.2.1) at the time and place of capture.

3.4.1 Warm-up rates

To determine the effect of wing morphology on warm-up and cooling rates, thoracic temperature measurements were taken using both live and freshly dead specimens (which have the same thermal properties as live butterflies (Watt 1968, Wasserthal 1975). The techniques used here were based on those developed over a number of years in studies of butterfly body temperature and warming rates (e.g. Watt 1968, Wasserthal 1975, Roland 1982, Guppy 1986b, Kingsolver 1985a, 1987a, Schmitz & Wasserthal, 1993).

Butterflies were tested using the following protocol: All experiments took place in an unheated room, at an ambient temperature (T_a) of $\cong$ 24°C (there was no significant difference in T_a for the different subsets of individuals tested (i.e. males and females, northern and southern butterflies). Individuals were killed (killing jar containing ethyl acetate) and then placed on a setting board with the wings fully open. The wings were held in this position with strips of setting paper pinned over the outer edge of the wing. Holding the wings in this way should not interfere with warm-up rates, as in dorsal basking only the basal 5 - 10 mm of wing is thought to have significant effect on heat transfer to the thorax (Watt 1968, Wasserthal 1975, Kingsolver & Moffat 1982,). A 0.4 mm diameter thermistor probe (Sky Instruments SKTS 200U/M) was inserted under the

cuticle of the dorsal thorax, held in place with a small blob of water soluble glue (Gloy™), and connected to a seven channel data logger (Sky Instruments SDL 2960), set to take readings at 30 second intervals. The data logger recorded simultaneous readings from a pyranometer, to record light levels (radiation in W m^{-2}), integral thermistor with radiation shield, to record ambient temperature, and a bare thermistor probe as a control.

Once the thoracic probe was inserted the butterfly was left in low light conditions (4 - 5 Wm^{-2}) for ten minutes. The butterfly, bare control probe and pyranometer were then placed under a 60 W light bulb, positioned 15 cm above the butterfly, for 10 minutes. The light was then switched off and the butterfly allowed to cool for a further 10 minutes.

3.4.2 Wing morphology

Digital image analysis (Windig 1991) was used to quantify the components of wing morphology which were of interest in this study. The sample of individuals used for quantifying wing morphology were those caught in 1996 in Bernwood Meadow and NW Scotland, a subset of which had been used in the warm-up experiments (see above).

Wings were removed from the butterflies and glued to cards, with one forewing and hindwing presented dorsally, the other ventrally (Figure 7). Digital colour images of the wings were made using a camera attached to a PC. Images were saved for subsequent analysis using Optimas™, a digital image analysis program.

Due to time constraints, only the dorsal forewings were used. The Optimas™ digital image analysis program was used to quantify the variables which were thought to be relevant to thermoregulation and flight efficiency: wing perimeter, length, breadth, area (i.e. wing size and shape) and basal grey value (luminance or "darkness" of the basal third of the wing, i.e. the area of relevance to thermoregulation), on a scale of 0 (black) to 255 (white).

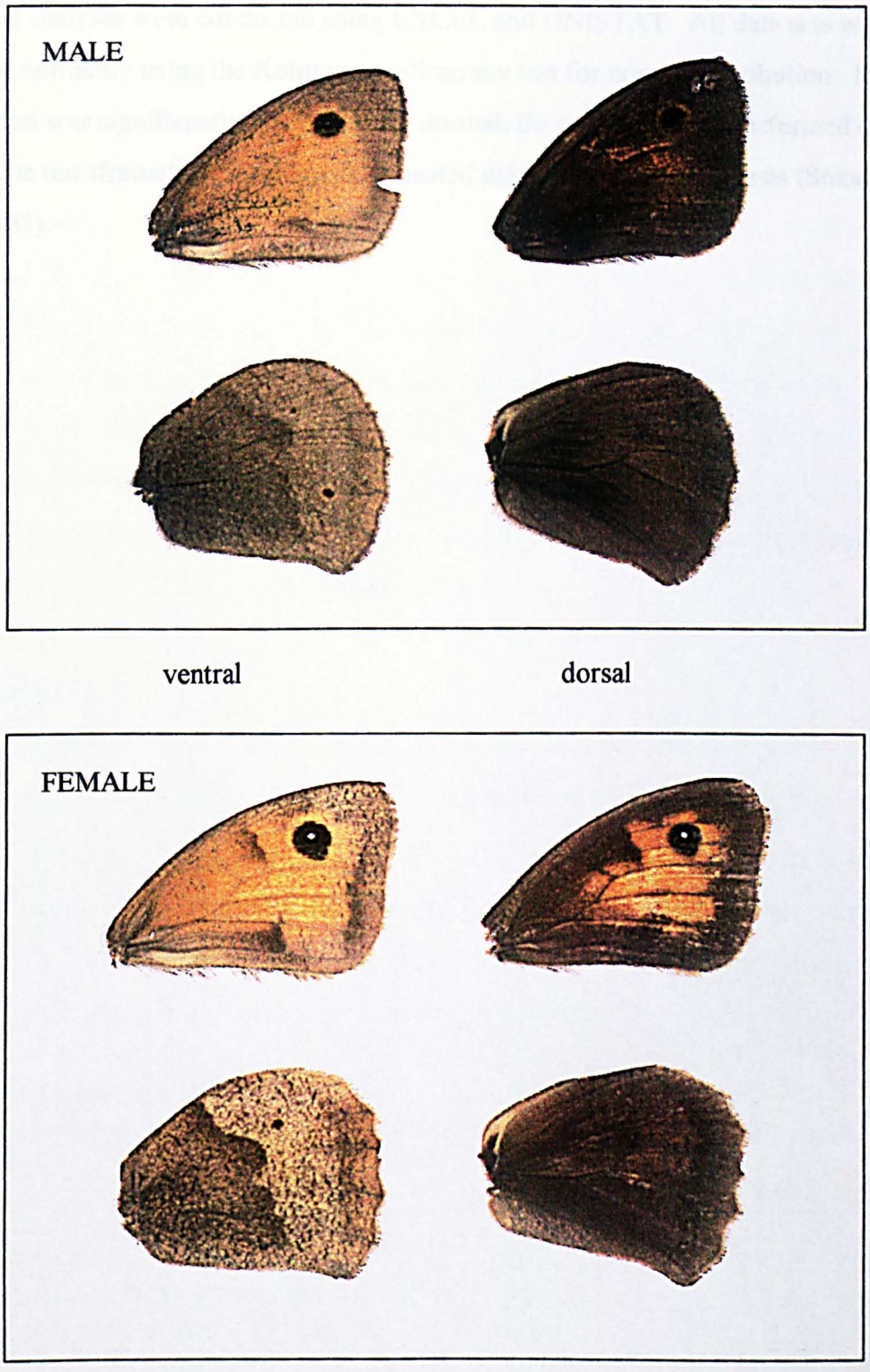


Figure 7. *Maniola jurtina*, wings mounted for digital image analysis

3.5 Data Analysis

Statistical analyses were conducted using EXCEL and UNISTAT. All data sets were tested for normality using the Kolmogorov-Smirnov test for normal distribution. If data distribution was significantly different from normal, they were either transformed ($\log_{10}$ and arcsine transformations were used) or treated using non-parametric tests (Sokal & Rohlf 1981).

CHAPTER 4. CLIMATE AND WEATHER

Much of the published data on butterflies and climate in temperate regions refers to mean temperatures and solar radiation for July. Latitudinal and geographic gradients in July temperatures have been shown to correlate with species abundance, diversity and morphological variation (Dennis & Williams 1986, Turner *et al.* 1987, Dennis & Shreeve 1989). In Britain the flight period of meadow browns in the south of England is from mid to late June through to late September or early October, with the peak flight period in July (Emmet & Heath, 1989). In north west Scotland, in the area where field work took place in this study, the flight period is much shorter with very few meadow browns flying before the end of June or after the end of August (Figure 8). All field work in Scotland took place in July. Data for general weather conditions in the southern and northern sites is therefore given for July.

4.1 Published data

Using data from 1961 to 1990, mean July temperatures for NW Scotland, at medium altitude, were approximately 3.5 °C cooler than those for around Oxford and the Chilterns (altitude of 79 m for England and 155 m for Scotland). (Data for the nearest 10 km^2 to where field work took place, from the Climatic Research Unit, University of East Anglia). The northern site experienced nearly two hours less sunshine each day, was windier and had approximately ten days more rain (Table 1).

Table 1. Climate data: July 1961 - 1990
Adapted from data supplied by UK Met. Office to Climate Research Unit, University of East Anglia.

	SC England	NW Scotland
minimum mean surface air temperature (°C)	11.2	9.1
mean mean surface air temperature (°C)	16.3	12.7
maximum mean surface air temperature (°C)	21.5	16.4
mean hours of sunshine day^{-1}	6.1	4.3
mean number of rainy days	10.9	20.6
mean wind speed (m sec^{-1}) at 10 m	3.8	5.2

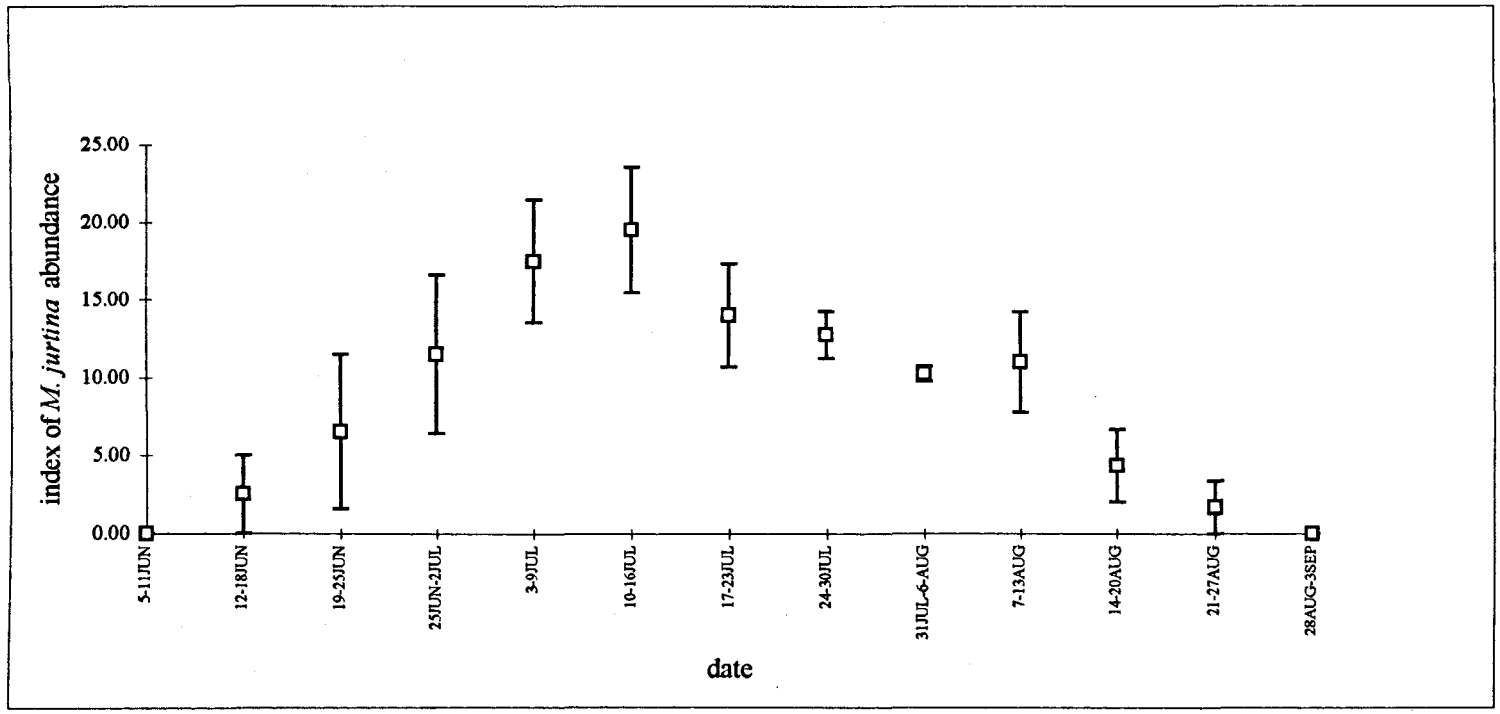


Figure 8. Meadow brown index of abundance (combined values for two transects) mean for 1989-1992. Ardtornish Estate, Morvern, Argyll.

data from Donald Kennedy, warden of Rahoy Hills Nature Reserve; transects walked for butterfly monitoring scheme.

4.2 Field work data

Both July 1994 and July 1995 were very hot summers. Conditions in NW Scotland in 1995 were unusually hot and dry, so the conditions under which the Scottish field work took place were atypical for that area and that time of year.

Values for environmental variables were recorded at the time and sites where field work took place. When analysing data for correlations between environmental variables it is inappropriate to use the raw data of readings taken every minute during field work, as this would involve data sets of many thousands of points, many of which are redundant (e.g. if conditions remain essentially unchanged for long periods of time). The data are therefore summarised into subsets, such as mean values for each transect walked or as mean values for the duration of each individual followed. Each subset is then used for analysis of different aspects of behaviour. For a general comparison between the conditions at the northern and southern sites during 1994 and 1995, combined data for transects and following individuals is used.

Ambient temperature in the south was approximately 1°C warmer than in the north, while levels of sunshine were very similar. Ground temperatures (T3) were approximately 1.5°C higher in the north and the northern site was generally much windier (Table 2).

Table 2. Mean values of environmental variables recorded during field work at southern (July 1994) and northern (July 1995) sites. (n = number of transects + number of individuals followed).

	SOUTH n = 169	NORTH n = 84	t	*P*
T_a (°C)	21.9 ± 0.2	20.8 ± 0.3	2.9	**
T2 (°C)	22.9 ± 0.3	21.1 ± 0.3	4.2	***
T3 (°C)	26.7 ± 0.4	28.3 ± 0.6	2.1	*
SR (Wm^{-2})	547.1 ± 18.5	527.9 ± 25.4	0.6	NS
wind ($m\ min^{-1}$)	32.8 ± 1.5	64.6 ± 2.4	11.1	***

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant

4.2.1 Correlations between environmental variables

When looking for correlations between environmental variables, results again depend on which sub-set of data is used. For a general overview of correlations between environmental variables at the two study areas, data obtained while following individual butterflies are used.

At both northern and southern sites there is a significant correlation between solar radiation and each of the three measures of temperature. The three measures of temperature are also correlated with each other. At the southern site there is a negative correlation between wind and both T_a and T2 (the "wind chill" factor). The results for the northern site are more difficult to interpret, with a positive correlation between wind and all three measures of temperature (Tables 3 and 4).

Table 3. Correlation between environmental variables, data from following individuals, southern population, July 1994 (n = 85).

	T_a (°C)		T2(°C)		T3(°C)		SR (Wm^{-2})	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
T2 (°C)	0.91	***						
T3 (°C)	0.22	*	0.31	**				
SR (Wm^{-2})	0.21	*	0.31	**	0.42	***		
wind (m min^{-1})	-0.58	***	-0.47	***	-0.1	ns	-0.13	NS

Table 4. Correlation between environmental variables, data from following individuals, northern population, July 1995 (n = 57).

	T_a (°C)		T2 (°C)		T3 (°C)		SR (Wm^{-2})	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
T2 (°C)	0.99	***						
T3 (°C)	0.89	***	0.90	***				
SR (Wm^{-2})	0.55	***	0.56	***	0.67	***		
wind (m min^{-1})	0.26	*	0.27	*	0.43	***	0.13	NS

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
NS = not significant

This general data on the different weather conditions in the two study areas provides a framework for subsequent more detailed analysis of the influence of environmental variables on meadow brown behaviour and morphology.

CHAPTER 5. DENSITY OF MEADOW BROWNS

5.1 Introduction

Critical factors influencing the range of a butterfly species are the availability of suitable larval food plants and physiological tolerances. Within a species range population size varies, spatially and temporally. In Britain many butterfly species (including *Maniola jurtina*) show a trend for decreasing population size towards the northern edge of their range, correlating with mean summer temperatures and levels of solar radiation (Dennis & Williams 1986, Turner 1986, Turner *et al.* 1987, Shreeve *et al.* 1996). The influence of these climatic variables may be indirect e.g. through their effect on larval food plant quantity and quality (Dennis 1993), competition for adult nectaring resources (Porter *et al.* 1992) or parasitism and predation (Rawlins & Lederhouse 1978, Bowers *et al.* 1985, Kingsolver 1987b, Warren 1992). More direct effects of weather on population size are through the influence of temperature and solar radiation on time available for flight (to locate mates, nectar sources and egg laying sites) and on realised fecundity (Stern & Smith 1960, Watt 1968, Kingsolver 1983a, 1985a, Dennis & Shreeve 1991, Shreeve 1992, Gossard & Jones 1997).

Three main questions are asked in relation to density of meadow browns in Oxford and Scotland:

1. Are the densities of male and female butterflies different at the northern and southern sites?
2. Does the ratio of male : female butterflies differ at the two sites?
3. Is the density of male and female butterflies correlated with T_a and SR?

5.2 Methods

Transect data are used to determine an index of density of meadow browns in the northern and southern regions. The index of density used here refers to "observed transect density", which is considered adequate for comparative purposes in this study. Individuals were counted if they were encountered within a 5m "box" while walking a transect (Pollard & Yates 1993) (chapter 3, section 3.2.3.1). Comparisons of density

between the northern and southern regions are made using data from transects walked in July, pooling results from within-region sites (27 transects for each region). Densities are given in contacts 100 m^{-1} (each 100 m (hm) of transect taking approximately 2.5 minutes to walk).

5.3 Results

5.3.1 Density of males and females in southern and northern populations

Meadow browns occurred at much higher density in the south than in the north. In the south, mean density of meadow browns encountered during transects was 3.47 hm^{-1}. In the north the density was only 0.65 hm^{-1}. Males occurred at significantly higher density than females in the south, but not in the north. Southern males occurred at significantly higher density than northern males, but there is no significant difference between the density of females in the north and south (Table 5). It is assumed that males and females were actually present with a 1:1 sex ratio, which is the case for most species of butterfly (Shreeve 1992). If this was the case then it is likely that females were under-recorded on transects because of differences in behaviour - i.e. they did not fly as frequently as males and so were less apparent.

Table 5. Mean density of meadow browns encountered during transects (butterflies hm^{-1})

	MALES	FEMALES	ALL
SOUTH	2.2 ± 0.37	1.3 ± 0.31	3.5 ± 0.65
NORTH	0.5 ± 0.10	0.2 ± 0.03	0.6 ± 0.11

Friedman Two-Way ANOVA : $\chi^2 = 26.28$, df = 3, $P = <0.0001$.

(Tukey-HSD was used to determine which pair-wise comparisons were significant).

5.3.2 Male : Female ratios in southern and northern populations

The mean ratio of males to females was determined, excluding transects on which no females had been recorded. The ratio of males : females was higher in the northern than in the southern population (NW Scotland = 6.29 ± 1.69, SC England = 2.36 ± 0.47), but the difference is not significant (Mann-Whitney U test: z = 0.60, n = 48, P = 0.54). It is assumed that males and females were actually present in a ratio of 1:1 and that the observed ratios were due to females being under recorded (see above).

5.3.3 Density and environmental variables

For the southern population there is a significant correlation between density of male (but not female) butterflies and solar radiation. For the northern population there is a significant correlation between both male and female butterflies and solar radiation. There is no significant correlation between density and ambient air temperature at either site (Table 6).

Table 6. Spearman's rank correlation coefficient: density of meadow browns (butterflies hm^{-1}) with T_a (°C) and solar radiation (Wm^{-2}), and between males & females (n = 27).

	T_a		SR		♂	
	r_s	P	r_s	P	r_s	P
SOUTH						
SR	0.49	**				
♂	0.31	ns	0.33	*		
♀	0.29	ns	0.04	ns	0.75	***
NORTH						
SR	0.65	***				
♂	0.07	ns	0.55	**		
♀	0.01	ns	0.38	*	0.58	**

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
ns = not significant

5.4 Discussion

Population size and recorded density of butterflies at any particular site may fluctuate from year to year, under the influence of a range of factors (e.g. food plant availability, parasitism and predation, variation in weather conditions – Brakefield 1987, Pollard & Yates 1993). For this reason, a one year comparison of population densities has obvious inherent limitations. However, the results presented here, for lower density of butterflies in the northern population, are consistent with trends found in other studies (Dennis & Williams 1986, Turner 1986, Turner *et al.* 1987, Shreeve *et al.* 1996).

Although most species of butterfly are expected to have an overall 1:1 sex ratio, there are cases reported where this ratio has been distorted (Porter 1984, Bowden 1987). However, the early emergence of males (protandry) may lead to an unequal ratio of adults early in the flight season. The most likely explanation for the higher density of males than females in the southern population is a combination of inclusion of data from early in the flight season (no females were present during transects walked at the end of June) and the differences in behaviour of males and females, with the former being more active (Brakefield 1982a, 1982b). This difference in activity between the sexes is also likely to be the main explanation for the male : female bias in the north.

The results clearly demonstrate the difference in overall density between northern and southern populations, at the time of this study. Availability of suitable food plants is unlikely to be the main limiting factor for meadow browns in the north, whereas temperature and solar radiation, through their influence on factors such as larval development and growth rates, time available for flight and fecundity are likely to be key factors in limiting population size (Shreeve *et al.* 1996).

Northern populations were also found to be more restricted in the habitats in which they occurred. When surveying for suitable study sites at the start of the Scottish field work, meadow browns were found to be absent from many areas of grassland, which would have almost certainly been suitable, had they been further south. The only sites where meadow browns were found were all on south facing slopes, which were warmer (and dryer). This conforms to the pattern for other species which may occupy broad niches

at the centre of their range, but are restricted to smaller areas with a warmer microclimate further north (Thomas 1993, Thomas *et al.* 1994).

Although long term monitoring of changes in population sizes was obviously not possible here, Thomas *et al.* (1994) report that many species, including *M. jurtina* , undergo greater fluctuations in population size towards the north of their range. This, together with the decreased flight season (Brakefield 1987, Kennedy *pers.com.*) all serve to make the adult butterflies and the population as a whole more vulnerable to stochastic and environmental perturbations, such as extremes of weather.

The low population density and greater isolation of the Scottish populations, existing in climatically marginal conditions, is likely to influence temporal persistence of populations, making them more vulnerable to local extinction and affecting the chances of recolonization (Hanski & Gilpin, 1991).

The correlation between transect density and solar radiation within each region is likely to be due to the influence of solar radiation on individual activity, with more butterflies engaged in active flight (and therefore recorded on transects) in sunny conditions. In warm, sunny conditions butterflies have been found to spend between 50% and 80% of their time in active flight (Kingsolver 1983a, 1983b, Heinrich 1986a, 1986b, Pivnick & McNeil 1987), whereas in cooler, cloudy conditions they are more likely to be resting or basking for a higher proportion of the time, and will consequently be under-recorded on transects. This again emphasises the importance of solar radiation intensity and hours of sunshine for time available for flight - an essential pre-requisite for locating mates, nectar sources and egg laying sites.

CHAPTER 6. BEHAVIOUR AND WEATHER CONDITIONS.

6.1 Introduction

During the course of their adult life, butterflies engage in a number of activities, which are essential for survival and reproduction. Flight is perhaps the most important activity, as for males this is a pre-requisite for predator avoidance, foraging and locating mates, and for females, predator avoidance, foraging and locating egg laying sites (Kingsolver 1985a, Shreeve 1992). In warm and sunny conditions, which are suitable for active flight, butterflies can allocate their time between activities such as foraging or mate location. In cooler and less sunny conditions a greater proportion of time may have to be spent in basking to achieve thoracic temperatures necessary for active flight. At low ambient temperatures (T_a) and solar radiation (SR) intensities, certain activities, such as active flight may be severely restricted or precluded (Rawlins 1980, Kingsolver 1983a, 1983b, Heinrich 1986a). It is expected that both the proportion of the population engaged in a particular activity, and the percent of time allocated to each activity will vary under different conditions of ambient temperature and solar radiation (Pivnick & McNeil 1987).

In marginal climatic areas, butterflies may show adaptations to increase thermoregulatory efficiency (Kingsolver 1983a, 1983b) which may lead to different patterns of activity, as may constraints imposed by habitat structure and population density. Differences in the behavioural ecology of males and females may also lead to different activity patterns for each sex (Brakefield 1982a).

Here, I present an overview of how activity patterns vary under different weather conditions, by investigating how the proportion of the population engaged in a particular activity and the percent of time allocated to each activity, varies under different conditions of ambient temperature and solar radiation. In subsequent chapters each category of behaviour is analysed in greater detail. Differences between males and females and between northern and southern populations are also discussed.

6.2 Methods

Data from transects and following individual butterflies are used to explore how behaviour varies under different weather conditions (different sections of "environmental space"). Pooled data for transects for each region (north and south) and for each sex are used to determine the percent of the population engaged in each category of behaviour under different weather conditions. Two environmental variables are used for this analysis; solar radiation and ambient air temperature. Each of these variables is divided into the quartiles of their range, to produce a map of environmental space with sixteen sections, corresponding to different weather conditions (Figure 9).

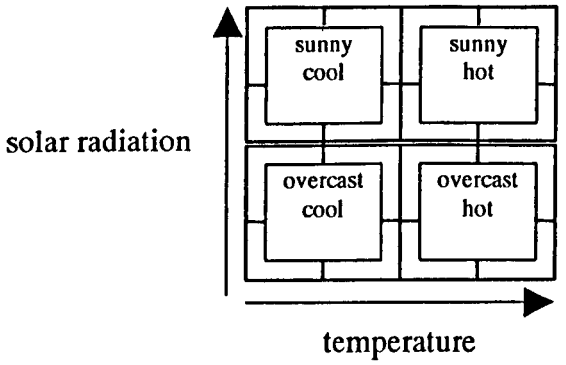


Figure 9. Map of environmental space.

For each sex, the percent of butterflies engaged in each category of behaviour is plotted, for each section of environmental space (e.g. male *M. jurtina* recorded flying, as a percent of all male *M. jurtina* recorded for transect conditions which fell into that section of environmental space).

A similar procedure is adopted for plotting time allocated to each category of behaviour under different weather conditions, with data obtained from following individual butterflies. For each area of environmental space, a value is derived for the total contact time for observations, which occurred under those weather conditions (for each sex, in each region). Duration of each category of behaviour, as a percent of total contact time, is then plotted.

6.3 Results

Figure 10 gives an overview of the differences in behaviour between males and females and between southern and northern regions. For each sex in each region, transect data are pooled to give the percent occurrence of each category of behaviour (all weather conditions). Data from following individuals give the overall percent of time allocated to each behaviour. These data represent the main trends in behaviour at the population level.

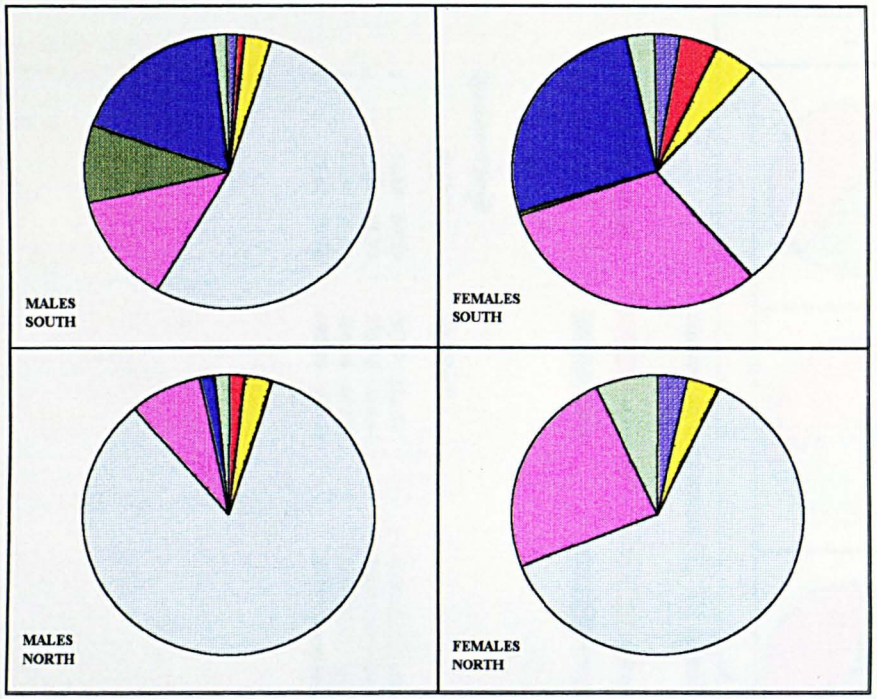
Plots of percent occurrence of each category of behaviour (transect data), for each of the sixteen sections of environmental space, are given in figures 10a-10d. Percent of time allocated to each behaviour (following individuals), for each of the sixteen sections of environmental space, is given in Figures 11a-11d. (The behavioural category "other" includes infrequently recorded behaviour such as mating, courtship, and miscellaneous behaviour such as a butterfly caught in a spider's web).

This method of presenting the data is useful for providing a quick visual assessment of overall trends and for comparing differences between the sexes and between the regions. More detailed statistical analysis of these trends and comparisons is given in subsequent chapters.

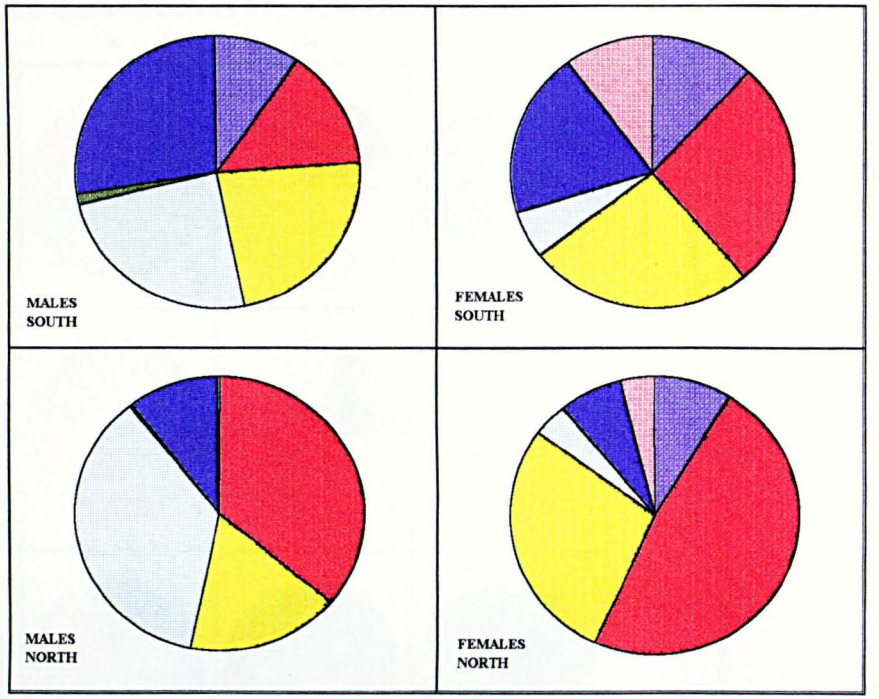
6.3.1 Percent occurrence of each behaviour

Examination of the transect data (percent occurrence of each category of behaviour, Figures 10a - 10d) shows that the behavioural category "flew when disturbed" (FWD) was recorded most frequently in cool and cloudy conditions, for both sexes in northern and in southern populations. When conditions were warmer and/or sunnier, this behaviour formed a smaller percent of the behaviours observed. Active flight was recorded under all weather conditions, but formed a higher percent of observed behaviour as temperature and solar radiation levels increased. Comparing males and females of the southern population, a higher percentage of males than females were recorded flying, under all weather conditions. In warm, sunny conditions, 58% of all males recorded were flying. For females the maximum percent of individuals recorded flying was 33%.

PERCENT OCCURRENCE OF EACH BEHAVIOUR - TRANSECT DATA



PERCENT OF TIME ALLOCATED TO EACH BEHAVIOUR - FOLLOWING INDIVIDUALS



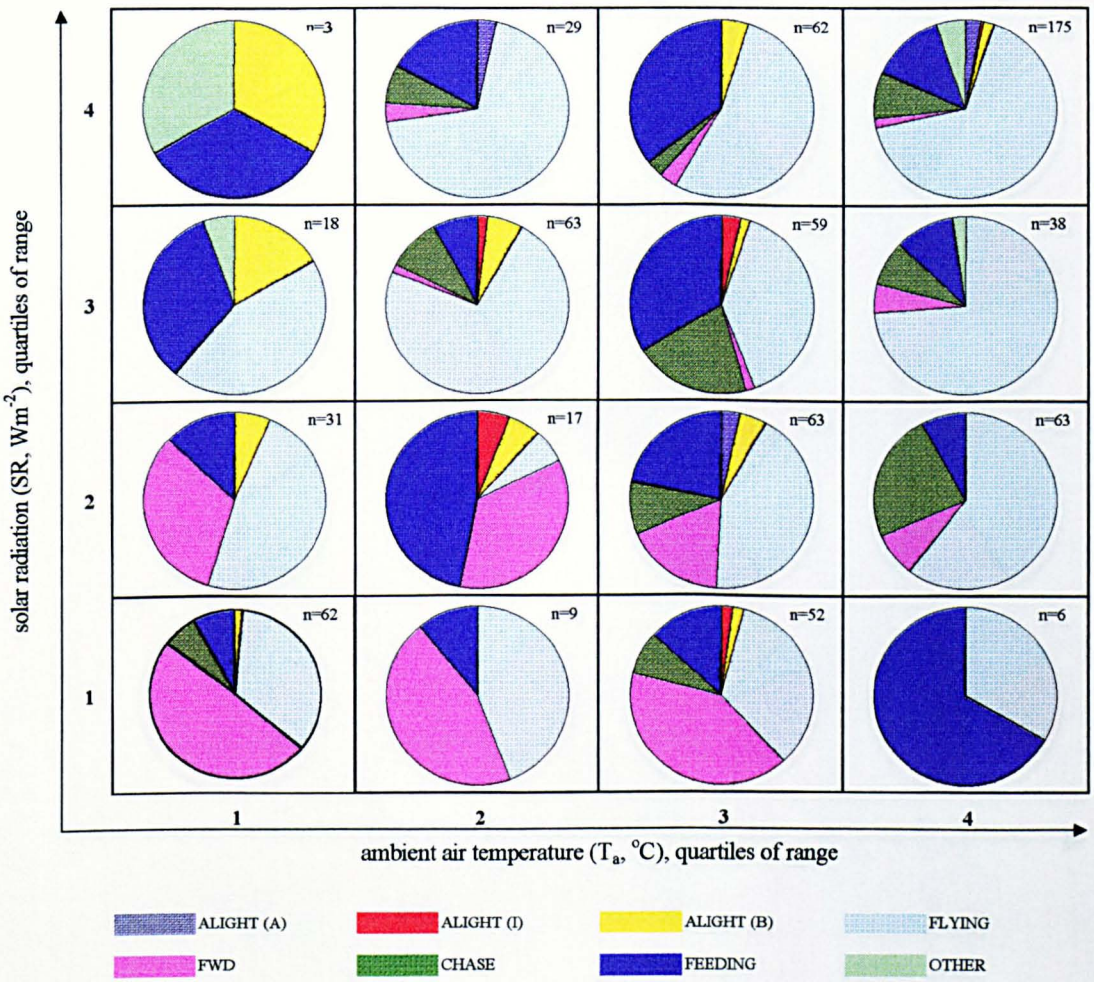
		MALES SOUTH		*FEMALES SOUTH*		*MALES NORTH*		*FEMALES NORTH*	
code	*behaviour*	*% occur*	*%time*	*% occur*	*%time*	*% occur*	*%time*	*% occur*	*%time*
	heat avoiding (alight A)	1.1	9.6	2.9	11.6	0.0	0.4	3.4	8.7
	intermediate posture (alight I)	0.8	14.1	4.0	27.3	1.6	35.3	0.0	48.0
	basking (alight B)	3.1	22.9	4.7	25.7	3.2	17.2	3.4	28.6
	flying	53.5	24.1	27.0	5.4	83.9	36.4	62.1	3.8
	chase	9.2	1.2	0.4	0.0	0.0	0.3	0.0	0.0
	feeding	17.9	27.8	26.7	19.6	1.6	10.2	0.0	6.9
	flew when disturbed (FWD)	13.1	-	31.0	-	8.1	-	24.1	-
	egg laying sequence	-	-	0.0	10.1	-	-	0.0	4.0
	other	1.6	-	3.1	-	1.6	-	6.9	0.0
	*N (all behaviours)**	748	701	445	443	62	489	29	678

% occur = % occurrence of each category of behaviour, pooled data from transects.
%time = percent of time allocated to each behaviour, pooled data from following individuals.
** N for % occur = total number of cantacts (all behaviours) on transects.*
N for % time = total number of contact-minutes (all behaviours) from following individuals.

Figure 10. All behaviour, under all weather conditions: Percent occurrence of each category of behaviour (transect data) and percent of time allocated to each behaviour (following individuals). *Maniola jurtina*, southern & northern populations.

Figure 10a. Percentage occurrence of each category of behaviour under different conditions of SR and T_a. Pooled data for transects (n = number of butterflies).

Male *M. jurtina*, southern population.



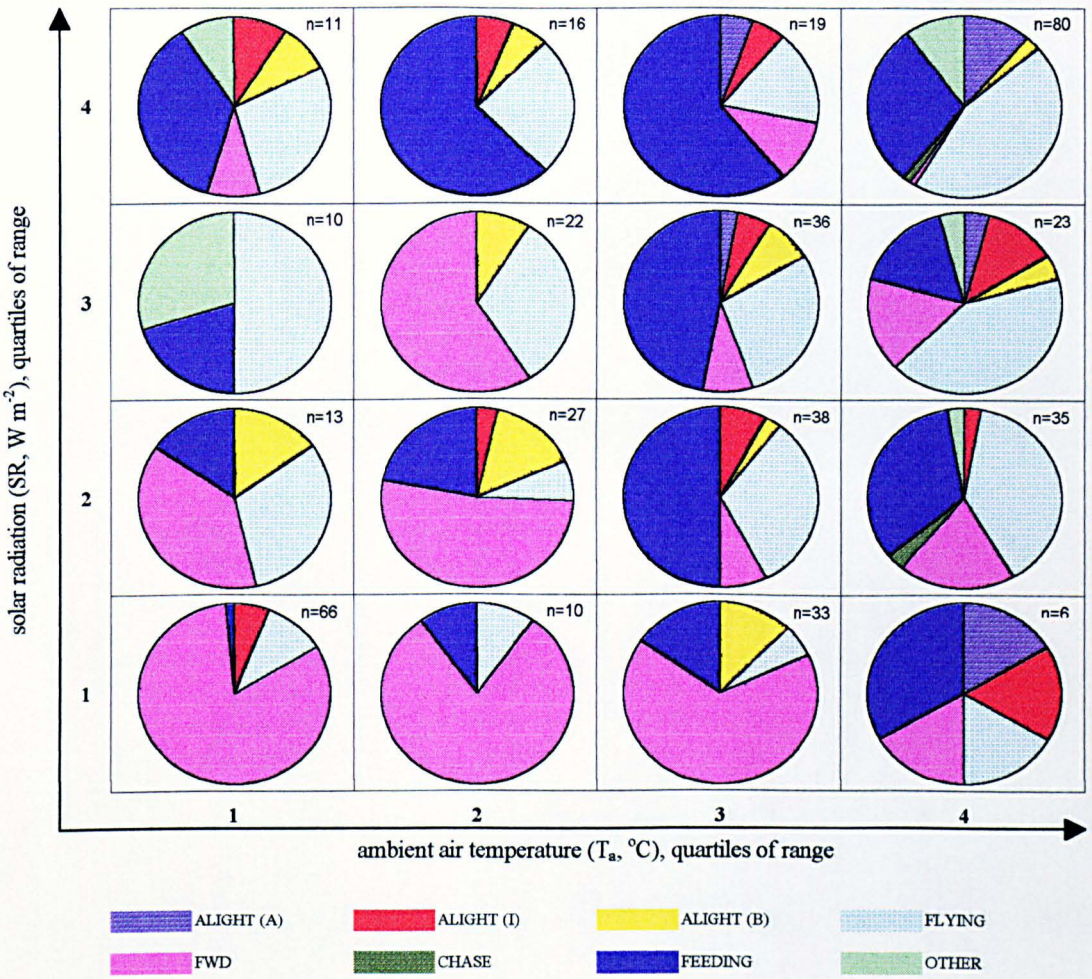
Quartiles of range

	T_a (°C)	SR (Wm^{-2})
1	14.65 - 19.59	96.99 - 272.54
2	19.60 - 21.33	272.55 - 466.64
3	21.34 - 23.41	466.65 - 692.95
4	23.42 - 29.26	692.96 - 924.78

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking
FWD = flew when disturbed

Figure 10b. Percentage occurrence of each category of behaviour under different conditions of SR and T_a. Pooled data for transects (n = number of butterflies).

Female *M.jurtina*, southern population.



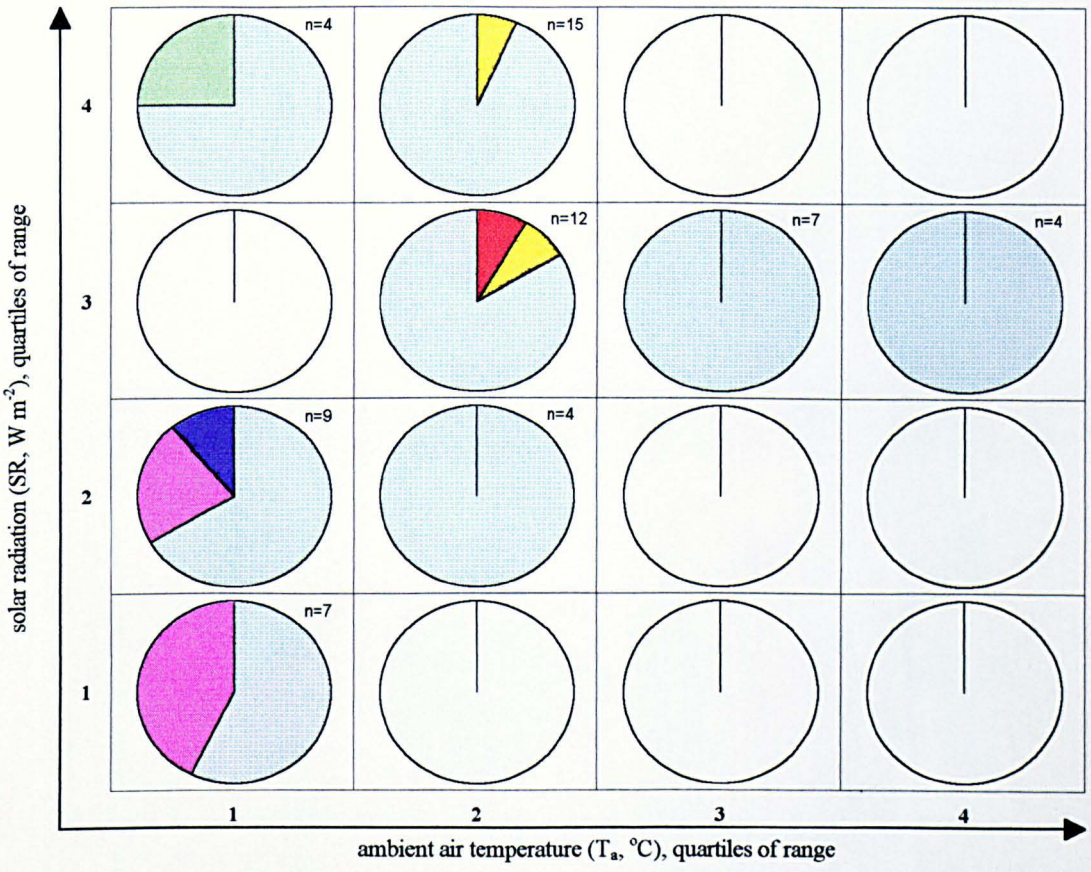
Quartiles of range

	T_a (°C)	SR (W m^{-2})
1	14.65 - 19.59	96.99 - 272.54
2	19.60 - 21.33	272.55 - 466.64
3	21.34 - 23.41	466.65 - 692.95
4	23.42 - 29.26	692.96 - 924.78

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking
FWD = flew when disturbed

Figure 10c. Percentage occurrence of each category of behaviour under different conditions of SR and T_a. Pooled data for transects (n = number of butterflies).

Male *M.jurtina*, northern population.



Empty circles = no data for these weather conditions.

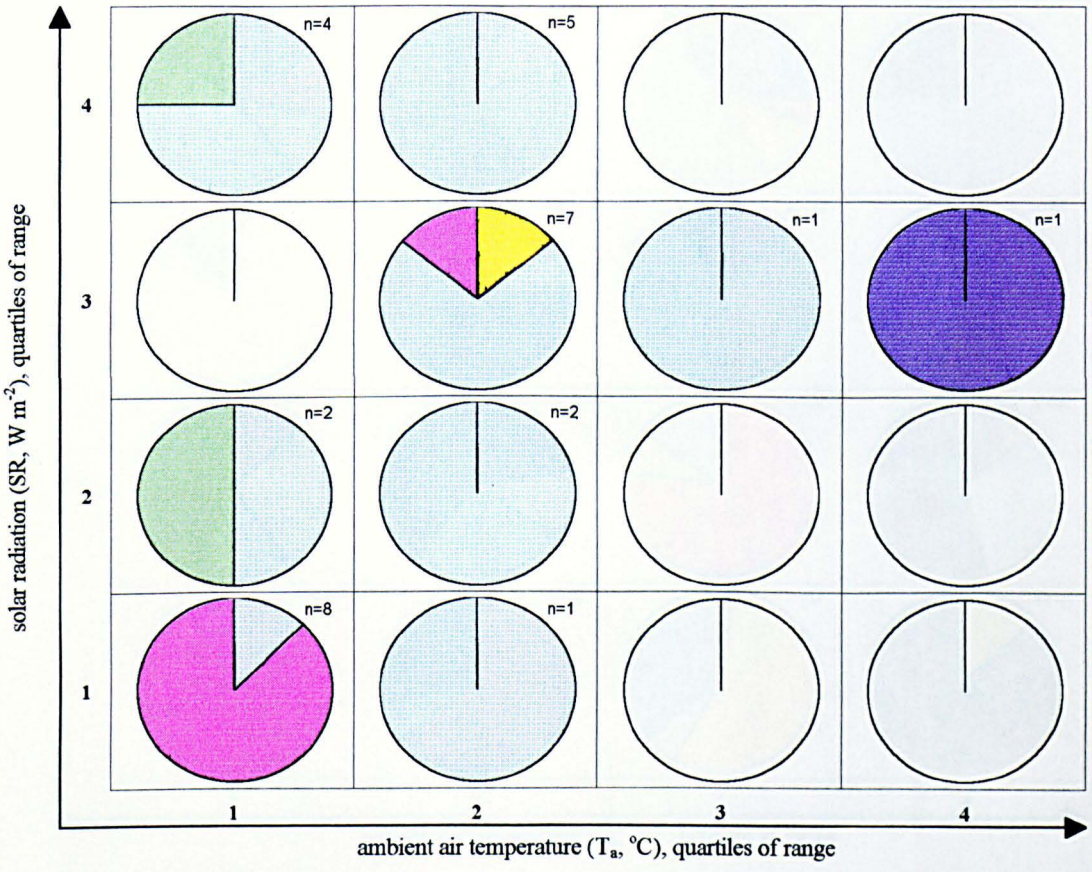
Quartiles of range

	T_a (°C)	SR (W m^{-2})
1	14.65 - 19.59	96.99 - 272.54
2	19.60 - 21.33	272.55 - 466.64
3	21.34 - 23.41	466.65 - 692.95
4	23.42 - 29.26	692.96 - 924.78

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking
FWD = flew when disturbed

Figure 10d. Percentage occurrence of each category of behaviour under different conditions of SR and T_a. Pooled data for transects (n = number of butterflies).

Female *M.jurtina*, northern population.



Empty circles = no data for these weather conditions.

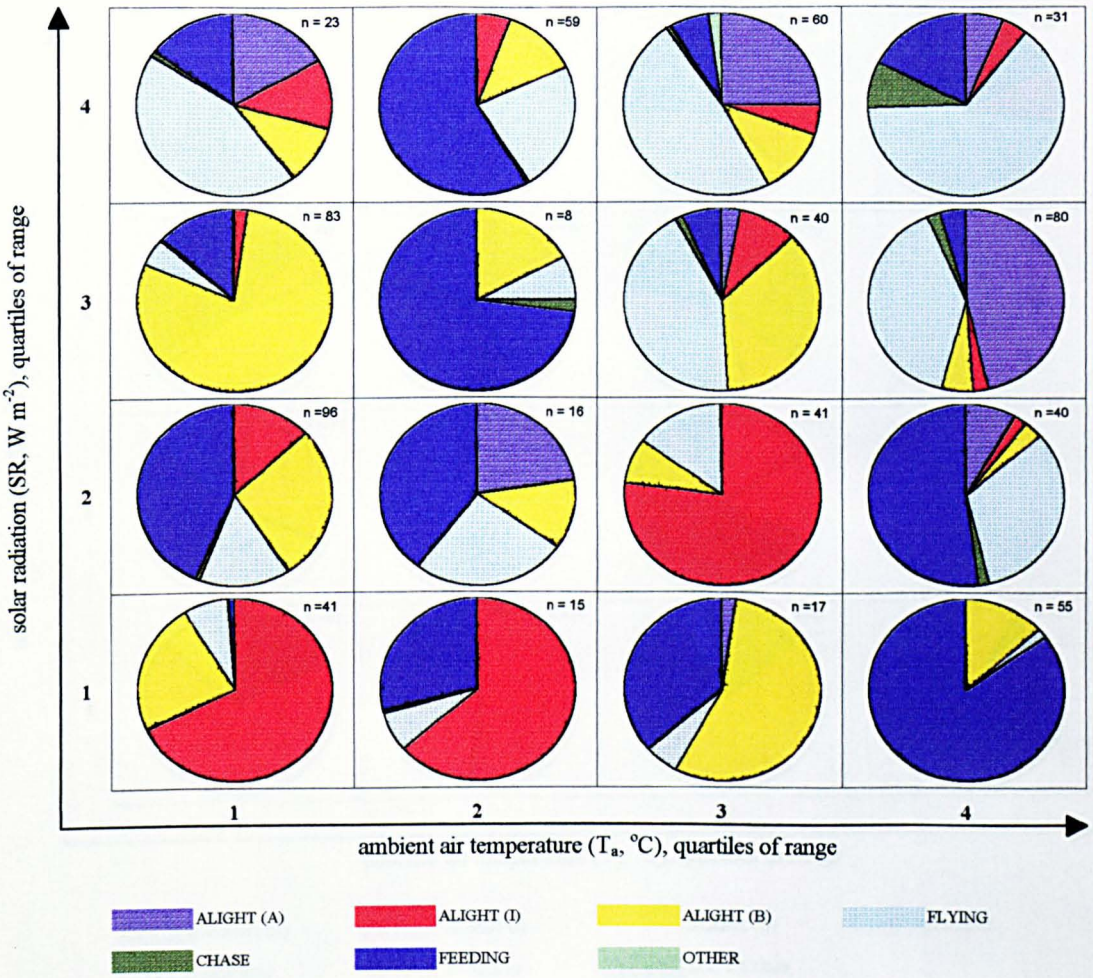
Quartiles of range

	T_a (°C)	SR (W m^{-2})
1	14.65 - 19.59	96.99 - 272.54
2	19.60 - 21.33	272.55 - 466.64
3	21.34 - 23.41	466.65 - 692.95
4	23.42 - 29.26	692.96 - 924.78

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking
FWD = flew when disturbed

Figure 11a. Percentage time allocated to each category of behaviour under different conditions of SR and T_a. Data from following individual butterflies (n = minutes of observation time)

Male *M.jurtina*, southern population.



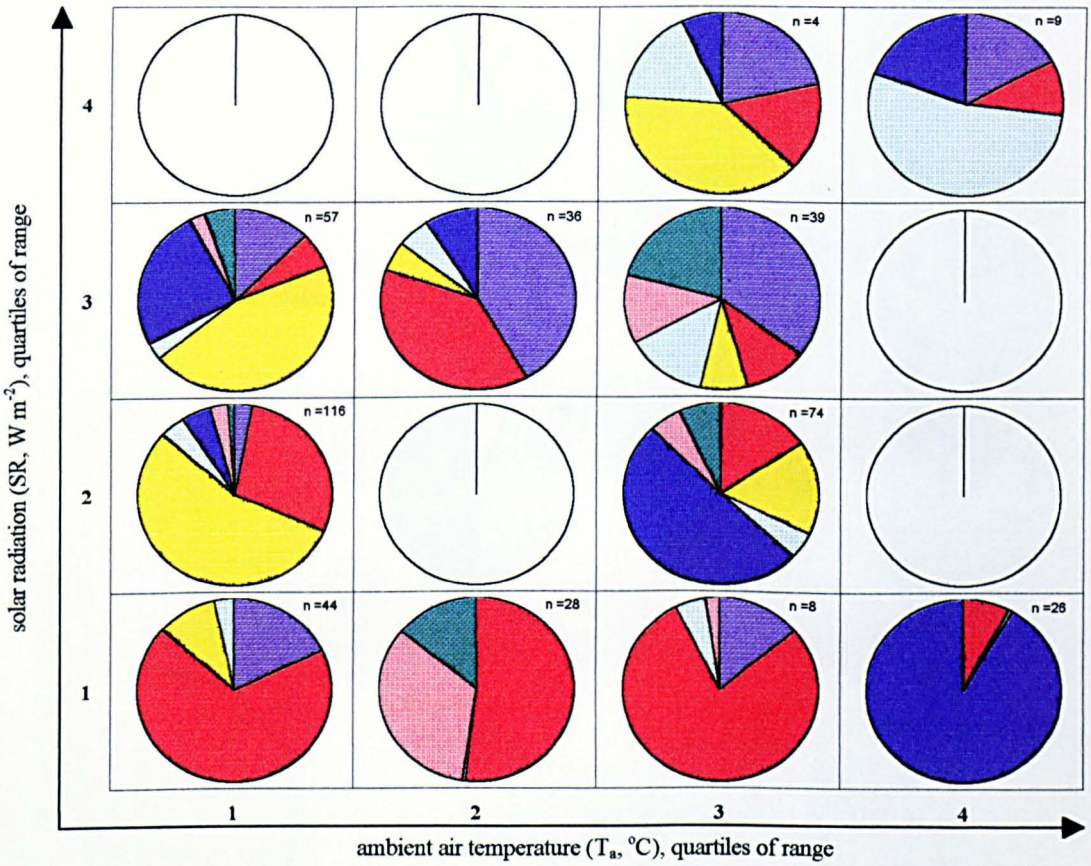
Quartiles of range

	T_a (°C)	SR (W m^{-2})
1	14.95 - 19.58	49.76 - 397.47
2	19.59 - 21.78	397.48 - 598.68
3	21.79 - 23.67	598.69 - 781.75
4	23.68 - 28.87	781.76 - 991.84

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking

Figure 11b. Percentage time allocated to each category of behaviour under different conditions of SR and T_a. Data from following individual butterflies (n = minutes of observation time)

Female *M.jurtina*, southern population.



empty circles indicate no data for these conditions

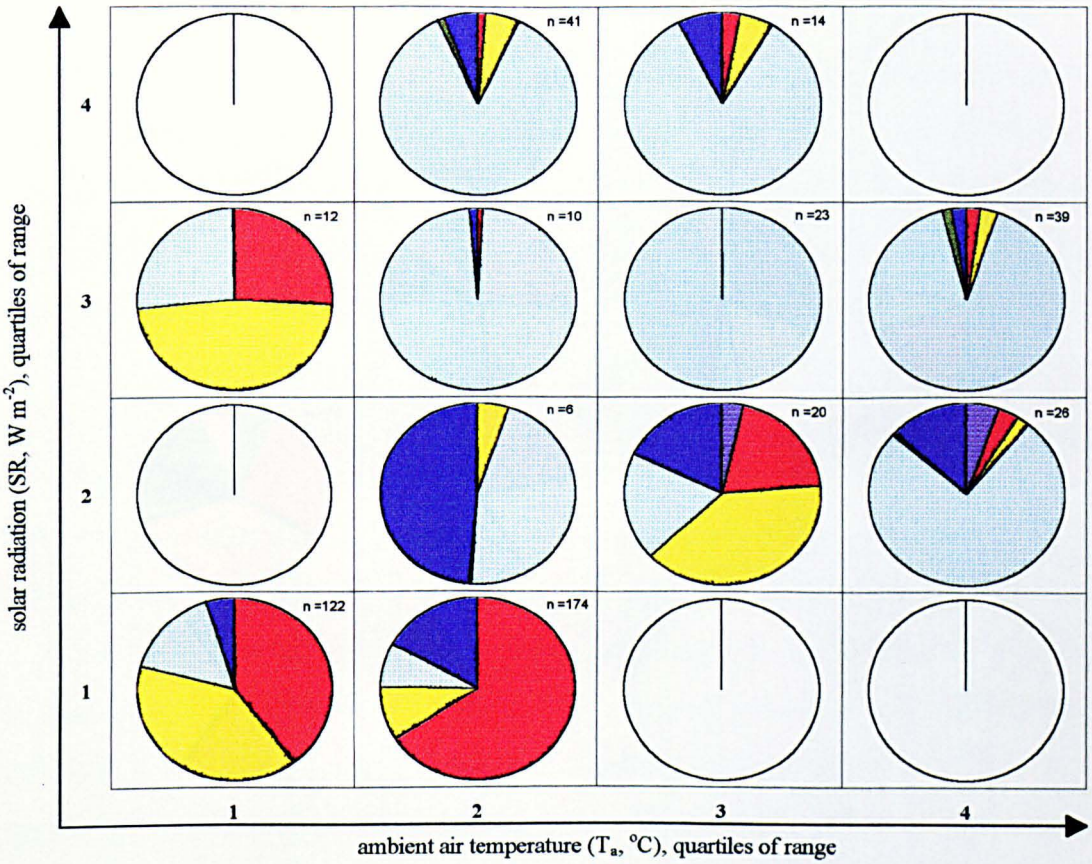
Quartiles of range

	T_a (°C)	SR (W m^{-2})
1	14.95 - 19.58	49.76 - 397.47
2	19.59 - 21.78	397.48 - 598.68
3	21.79 - 23.67	598.69 - 781.75
4	23.68 - 28.87	781.76 - 991.84

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking

Figure 11c. Percentage time allocated to each category of behaviour under different conditions of SR and T_a. Data from following individual butterflies (n = minutes of observation time)

Male *M.jurtina*, northern population.



ALIGHT (A) ALIGHT (I) ALIGHT (B) FLYING

CHASE FEEDING

empty circles indicate no data for these conditions

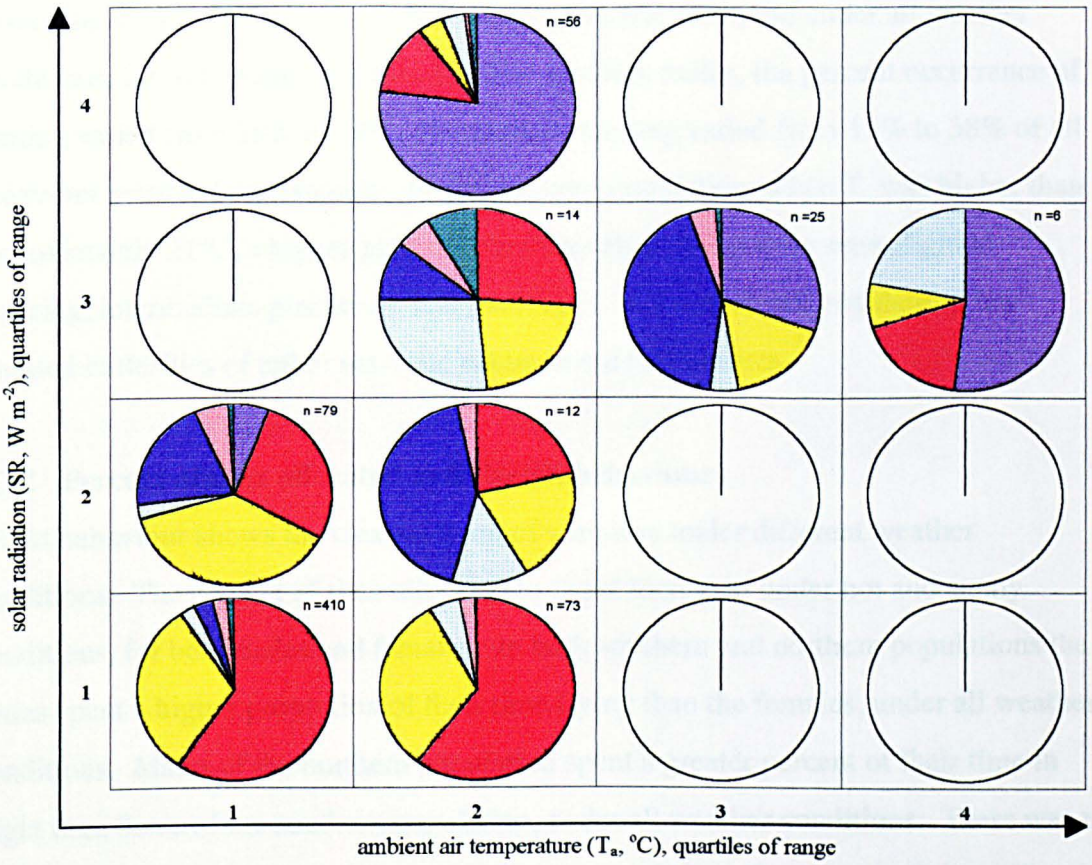
Quartiles of range

	T_a (°C)	SR (W m^{-2})
1	14.95 - 19.58	49.76 - 397.47
2	19.59 - 21.78	397.48 - 598.68
3	21.79 - 23.67	598.69 - 781.75
4	23.68 - 28.87	781.76 - 991.84

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking

Figure 11d. Percentage time allocated to each category of behaviour under different conditions of SR and T_a. Data from following individual butterflies (n = minutes of observation time)

Female *M.jurtina*, northern population.



empty circles indicate no data for these conditions

Quartiles of range

	T_a (°C)	SR (W m^{-2})
1	14.95 - 19.58	49.76 - 397.47
2	19.59 - 21.78	397.48 - 598.68
3	21.79 - 23.67	598.69 - 781.75
4	23.68 - 28.87	781.76 - 991.84

ALIGHT (A) = heat avoiding
ALIGHT (I) = intermediate posture
ALIGHT (B) = basking

Data for transects in the northern population must be treated with caution, as the numbers of butterflies recorded on transects was small. However, the majority of butterflies encountered on transects in the northern population were recorded in active flight, over a wide range of weather conditions for which data are available.
Few incidences of feeding were observed in the northern population during transect observations. In the southern population, feeding was observed under all weather conditions, for both males and females. For southern males, the percent occurrence of feeding varied from 20% to 28%. For females feeding varied from 12% to 38% of all behaviour recorded on transects. In the southern population, when T_a was higher than approximately 21°C, a higher percent of females than males were seen alighted (basking, intermediate posture or heat avoiding). In the northern population, few alighted butterflies of either sex were encountered on transects.

6.3.2 Percent of time allocated to different behaviours

Flight behaviour shows the clearest trend of variation under different weather conditions. The percent of time allocated to flight increased under hot and sunny conditions, for both males and females. In both southern and northern populations the males spent a higher proportion of their time flying than the females, under all weather conditions. Males of the northern population spent a greater percent of their time in flight than those of the southern population, under all weather conditions. There was no clear difference between northern and southern populations for percent of time allocated to flight by females.

6.3.3 Comparison of data from transects and following individuals

Presenting the data in this way helps to highlight the differences between the two methods of behavioural observation employed in this study - walking transects and following individuals. Comparing figures 10a and 11a it becomes clear that certain categories of behaviour may be under recorded or absent if only one of the two methods is employed. The behavioural category "flew when disturbed" (FWD - butterflies engaging in short "escape" flights when disturbed and landing again after a few metres) is not recorded when following individual butterflies. When following individual butterflies I maintained a distance of a few metres from the butterfly and tried to ensure

that I did not disturb the butterfly once it has alighted. This means that butterflies which would be recorded as "alighted" (basking, heat-avoiding or other) when following individuals, may well be recorded as FWD if encountered on a transect. Butterflies which are alighted are almost certainly under-recorded on transects, as they are less apparent than butterflies engaged in behaviour such as flight.

Other categories of behaviour which are absent or under recorded on transects are the walking and egg laying behaviour of females. This typically involves a sequence of behaviour with the females walking about low in the grass, laying an egg, walking again, laying another egg etc. (see Chapter 11). In cool cloudy conditions, for example, no females engaged in this behaviour were observed on transects. When following individuals under the same weather conditions, females spent a mean of 14% of their time walking and laying eggs.

6.4 Discussion

These data illustrate the constraints placed upon butterfly activity by low ambient temperatures and solar radiation intensity. In cool, cloudy conditions short "escape flights" (FWD), rather than active flying were recorded for a large proportion of the transect records, especially for female butterflies. Data from following individual butterflies demonstrates that these butterflies were probably alighted, in an intermediate posture (not basking or heat avoiding) prior to being disturbed. Although a proportion of the population was still engaged in active flight, data from following individual butterflies indicates that only a small proportion of time was allocated to this activity in cool, cloudy conditions. These conditions not only restrict activities, which are dependent on active flight, such as mate location, but also increase the likelihood of predation (Lederhouse 1983, Bowers *et al.* 1984). The fact that some individuals were still active in cooler, low solar radiation conditions suggests that there may be inter-individual variation in thermoregulatory efficiency and hence activity under different conditions of T_a and SR (Roland 1982, Kingsolver 1983a, 1983b, Heinrich 1993, Van Dyck *et al.* 1997a, 1997b).

Under warmer, sunny conditions a larger proportion of the population was engaged in active flight and a higher proportion of the time was allocated to active flight and flight related activities, such as "chasing". Shreeve (1984), in his study of *P. aegeria*, found that the proportion of observed individuals which were flying increased as air temperatures increased between 16°C and 22°C, and then remained stable at 65% for males and 45% for females.

In this study, the percent of the population engaged in basking and the proportion of time spent basking also decreased at higher ambient temperatures (e.g. see figures 11a – 11d). Heinrich (1986a) found that *Coenonympha inornata* males spent 73% of their time basking and only 23% of their time in active flight at ambient temperatures of 16°C. At high ambient temperatures they spent over 80% of their time flying, mostly in connection with searching for mates. Pivnick & McNeil (1987) report that *Thymelicus lineola* is almost inactive at low ambient temperatures and solar radiation of <100 Wm^{-2}. As T_a and SR increased basking was the main activity for both male and female *T. lineola*. At high T_a and SR males spent their time feeding and flying, in equal proportions, whereas females spent most of the time feeding.

The difference between the sexes in percent of the population engaged in active flight and the greater proportion of time allocated to flight by male *M. jurtina*, confirms similar findings for meadow browns by other workers (Pollard 1981, Brakefield 1982a). This reflects the different requirements of male and female butterflies. Female *M. jurtina* mate once, shortly after eclosion, and then tend to avoid males, flying mainly to feed or lay eggs. This means that for males, receptive females are a scarce resource and one of the main objectives of male flight is to find them. Brakefield also suggests that females spend more time feeding than males, but this distinction is not apparent here.

It is also possible that there are differences in thermoregulatory efficiency between males and females. The darker males may warm-up faster or achieve higher steady state temperatures than females, allowing them to become active at lower T_a and SR. Difference in thermoregulatory efficiency might also contribute to the increased flight

activity of northern compared to southern males - an hypothesis which is explored in subsequent chapters.

Egg laying was observed over a range of T_a, in both northern and southern populations, but not in the warmest conditions (T_a > 23.4 °C) for either population. Overall, females in the southern population allocated a greater percent of their time to egg laying.

Kingsolver (1983b, 1985a) argues that weather-related limitations on activity are a key factor in population dynamics for butterflies. The influence of weather is primarily through its effect on thermoregulation and flight activity and hence predation and realised fecundity (time available to lay eggs). A map of behaviour in relation to environmental space, as presented here, helps to highlight the limitations that weather imposes on different activities, and the differences in weather-related activity patterns between sexes and between geographic areas.

CHAPTER 7. ACTIVE FLIGHT

7.1 Introduction

The small body size of butterflies and their reliance on external sources of energy (primarily solar radiation) to achieve the temperatures required for active flight means that meteorological variation on a very short time scale (< 60 s) can have important implications for body temperature and hence flight activity (Kingsolver & Watt 1983).

In very cool conditions (ambient air temperature below 10 - 11°C) with low solar radiation intensity, butterflies are usually inactive (Heinrich 1986a, Pivnick & McNeil 1987). In slightly warmer conditions (T_a of 12 - 13°C) thoracic temperatures may be high enough for short duration flights (escape flights), but vigorous flight usually requires minimum ambient air temperatures of between 13°C (sunny conditions) and 17°C (shady conditions) (Pollard & Yates 1993). To maintain vigorous flight, thoracic temperatures (T_{th}) are usually in the region of 33 - 38°C (Kingsolver 1985a). At very high air temperatures and solar radiation intensities overheating may become a problem, reducing flight activity (Rawlins 1980, Roland 1982, Kingsolver & Watt 1983, 1984). A number of studies have investigated flight activity, under different environmental conditions, in species living at high latitudes and/or altitude (e.g. Kevan and Shorthouse 1970, Kingsolver 1983b, Kingsolver & Watt 1983, Heinrich 1986b), where conditions may be marginal for activity for much of the time, so the effect of variation in temperature and solar radiation is particularly pronounced. Kevan & Shorthouse, for example, looked at the activity of five species of butterflies in NorthWest Canada, and found that these butterflies were only active on sunny days. These butterflies also restricted their activity to areas with warm microclimates within their habitats.

Here, data from transects and from following individual butterflies are used to address a number of questions relating to flight behaviour of *M. jurtina*. The aims are to investigate the influence of environmental variables on flight behaviour and to determine whether there are any differences between flight activity of males and females and between the northern and southern populations.

The main questions asked are:

1. What are the minimum levels of ambient temperature and solar radiation for which flight was recorded, and do these differ for northern and southern populations?
2. What are the maximum levels of wind speed for which flight was recorded, and do these differ for northern and southern populations?
3. Is there a difference between northern and southern populations for the conditions under which flight was initiated? More specifically, do butterflies from the northern population initiate active flight at lower ambient temperatures and/or solar radiation levels than those from the southern population?
4. How is flight duration influenced by environmental variables?
5. Is there a difference in flight duration and the percent of time spent in flight between northern and southern populations?
6. Do males and females differ in flight frequency and duration, or conditions required for flight?

7.2 Methods

The field methods used to determine flight duration, frequency and associated environmental variables are given in Chapter 3, section 3.2. "Active flight" is used to describe butterflies which were flying vigorously, rather than the short escape flights (FWD), when butterflies would only fly for a few metres before landing again. Very short duration/distance flights (e.g. fluttering between flower heads on a single plant) and the walking/fluttering behaviour associated with egg laying are also excluded from this analysis.

7.3 Results

7.3.1. Minimum levels of ambient temperature and solar radiation for active flight.

Data from transects is used to determine the minimum levels of ambient temperature (T_a), solar radiation (SR) and the maximum wind speed (WS) at which active flight was recorded. For each variable data are given for the individuals for which the lowest T_a and SR, and maximum WS values were recorded for each site. Mean values were

determined by taking the lowest T_a and SR, and maximum WS for which active flight was recorded on each transect.

Ambient temperature

The minimum T_a for which active flight was recorded is 14.5°C for males and females in the southern population. The lowest temperatures for the northern population are 14.8°C for a male *M. jurtina* and 19.0°C for a female.

The mean of minimum T_a ($\bar{x}$ min T_a) for active flight is taken from the minimum T_a for which active flight was recorded, for each transect-day (all the transects walked on one day; total 23 days for south and 9 days for north). This is then used for comparison between northern and southern populations. For male *M. jurtina* the $\bar{x}$ min T_a is 20.8°C for the south and 19.9°C for the north. For females the values are 21.0°C and 20.2°C for south and north respectively. There is no significant difference between the values for north and south for either sex, or between males and females within each region. To see whether the minimum T_a for active flight merely reflects the weather conditions prevailing at the time, the minimum T_a for each transect as a whole was determined. There is no significant difference between any of the $\bar{x}$ min T_a values for active flight and the $\bar{x}$ min T_a values for the transects as a whole.

These results indicate that, during this study, the ambient temperatures recorded for both northern and southern sites were, on average, high enough for active flight to occur for some individuals of both sexes. The atypically warm summers of both 1994 and 1995 probably contributed to this. However, data presented in the previous chapter shows that at low T_a, only a small proportion of the population engage in active flight and the proportion of time allocated to active flight is small.

Solar radiation

The values for minimum levels of solar radiation (SR) for which active flight was recorded were determined in the same way as for T_a. The lowest levels of SR for active

flight for male *M. jurtina* are 19.5 Wm^{-2} and 150.0 Wm^{-2} for southern and northern butterflies respectively. For female *M. jurtina* the values are 67.2 Wm^{-2} and 170.0 Wm^{-2} for southern and northern butterflies respectively.

There is no significant difference between the northern and southern populations, for either sex, or between males and females within regions for the $\bar{x}$ min SR values for active flight. The $\bar{x}$ min SR values for active flight for northern *M. jurtina* are significantly higher than those recorded for the transects as a whole, for both male and female butterflies. For the southern population, the $\bar{x}$ min SR for active flight for females is significantly higher than values for transects as a whole, but there is no significant difference between $\bar{x}$ min SR values for males and those for transects as a whole (Table 7).

These results indicate that both males and females in the northern population were not able to engage in active flight over the whole range of solar radiation conditions available to them, but were only engaging in active flight when solar radiation intensity was higher than the average for transects as a whole. In the southern population, although there is no significant difference between males and females in the $\bar{x}$ min SR values for active flight, the females had a tendency to engage in active flight at the sunnier end of the range of conditions available to them (as indicated by the higher $\bar{x}$ min SR values for active flight in females than those for the transects as a whole).

Table 7. Minimum levels of solar radiation (Wm^{-2}) on transects and for active flight recorded for *M. jurtina* in northern and southern populations, with two-tailed t-tests, assuming unequal variance.

	n	*mean ± s.e.*	*comparison*	*t*	*P*
transects - south	23	224.1 ± 36.3	transect S - transect N	0.32	ns
transects - north	9	206.2 ± 45.4	transect S - ♂ south	1.42	ns
♂ south	23	295.2 ± 34.3	transect S - ♀ south	2.13	*
♀ south	16	359.6 ± 55.7	transect N - ♂ north	2.50	*
♂ north	8	440.7 ± 85.7	transect N - ♀ north	2.88	*
♀ north	7	480.3 ± 91.4	♂ south - ♀ south	0.04	ns
			♂ north - ♀ north	0.32	ns
			♂ south - ♂ north	1.58	ns
			♀ south - ♀ north	1.13	ns

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
ns = not significant

7.3.2 Maximum wind speeds for active flight

Maximum wind speeds at which active flight was recorded were determined in the same way as for T_a and SR. The max. wind for active flight in the southern population is 124.3 m min^{-1} for male *M. jurtina* and 113.1 m min^{-1} for females. The values for the northern population are 137.9 m min^{-1} for males and 116.5 m min^{-1} for females.

Northern males and females both flew in significantly windier conditions than those from the southern population, and there is no significant difference between $\bar{x}$ max. wind for males and females in Scotland. In Scotland $\bar{x}$ max. wind for active flight for males and females is also significantly lower than values recorded for transects as a whole. In the southern population there is no significant difference between $\bar{x}$ max. wind for males and females, and both males and females flew at significantly lower wind speeds than those recorded for transects as a whole (Table 8).

Table 8. Maximum wind speeds (m min^{-1}) on transects and for active flight; *M. jurtina* northern and southern populations.

	n	*mean ± s.e.*	*comparison*	*t*	*P*
transects - south	23	69.0 ± 6.6	transect S - transect N	6.01	***
transects - north	9	120.4 ± 5.3	transect S - ♂ south	2.29	*
♂ south	23	49.3 ± 5.4	transect S - ♀ south	2.06	*
♀ south	16	49.0 ± 6.7	transect N - ♂ north	1.76	*
♂ north	8	97.4 ± 12.5	transect N - ♀ north	3.34	**
♀ north	7	80.8 ± 11.6	♂ south - ♀ south	0.04	ns
			♂ north - ♀ north	0.96	ns
			♂ south - ♂ north	3.52	**
			♀ south - ♀ north	2.37	*

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
ns = not significant

These results indicate that butterflies in the northern population were found flying at higher wind speeds than those in the south, although in both regions butterflies avoided flying at the windier end of conditions available to them. This was the case even for the southern population where wind speeds were significantly lower, than those routinely found at the northern site.

7.3.3 Flight initiation

Data from following individual butterflies are used to determine the mean values of environmental variables under which active flight was initiated for each individual. At both northern and southern sites, males initiated flight at slightly higher temperatures and levels of solar radiation than females (Table 9).

Table 9. Mean environmental variables for initiation of active flight.

	n	T_a (°C)	T2 (°C)	T3 (°C)	SR (W m^{-2})	WIND (m min^{-1})
♂ south	62	22.2 ± 0.3	23.3 ± 0.7	26.7 ± 0.6	646.8 ± 25.2	27.9 ± 2.1
♀ south	23	20.8 ± 0.7	21.5 ± 0.6	25.7 ± 0.6	529.6± 53.6	35.2 ± 5.2
♂ north	34	21.7 ± 0.4	22.0 ± 0.4	30.6 ± 1.0	584.5 ± 34.6	67.3 ± 4.2
♀ north	20	20.8 ± 0.5	21.16 ± 0.51	28.6 ± 1.2	581.2 ± 53.2	60.7 ± 4.3

In the southern population the difference between SR levels for flight initiation for male and female *M. jurtina* approaches statistical significance ($t = 1.98, P = 0.056$), as does the difference in T_a between males and females ($t = 1.94, P = 0.056$). Females in the southern population initiated flight in conditions with significantly higher wind levels than did males ($t = 3.08, P = 0.004$). In the northern population there were no significant differences between males and females for any of the environmental variables associated with flight initiation.

When comparing the northern and southern populations, the only significant differences were for T3 (ground temperature) and wind, with both males and females from the northern population initiating flight at higher wind speeds and higher ground temperatures than those from the southern population (Table 10).

Table 10. Differences in wind speed and ground temperature associated with flight initiation in southern and northern populations of *M. jurtina*. (two-tailed t-tests, assuming unequal variance).

	t	*P*
wind (m min^{-1})		
♂ south - ♂ north	8.47	***
♀ south - ♀ north	2.30	*
T3 (ground temp) (^{o}C)		
♂ south - ♂ north	3.47	***
♀ south - ♀ north	2.17	*

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
ns = not significant

When comparing the conditions under which flight was initiated for northern and southern population, again, one confounding factor is the overall differences in environmental conditions between the two regions. It is therefore necessary to test whether the conditions under which flight was initiated reflect the overall conditions which were prevalent in the region. Comparisons were made using mean values for environmental variables from following individuals, for all behaviour, compared with the recorded values for flight initiation. There is no significant difference between these values, for any of the environmental variables, for either the southern or the northern population (Table 10a).

Table 10a. Comparison of mean values of environmental variables recorded for flight initiation with "overall" values for the region (values for all behaviours). (two-tailed t-tests, assuming unequal variance).

SOUTHERN POPULATION

	all behaviours	flight initiation males	flight initiation females
Ta (^{0}C)	21.79	22.25	20.77
T2 (^{0}C)	23.16	23.3	21.49
T3 (^{0}C)	26.41	26.69	25.74
SR (W m^{-2})	603.39	646.8	529.59
wind (m min^{-1})	33.19	27.94	35.2

NORTHERN POPULATION

	all behaviours	flight initiation males	flight initiation females
Ta (^{0}C)	21.17	21.73	20.78
T2 (^{0}C)	21.45	22.05	21.16
T3 (^{0}C)	29.15	30.6	28.6
SR (W m^{-2})	549.21	584.52	581.24
wind (m min^{-1})	63.96	67.3	60.71

COMPARISON OF FLIGHT INITIATION WITH ALL BEHAVIOURS

	SOUTHERN POPULATION						*NORTHERN POPULATION*					
	males			females			males			females		
	t	df	P	t	df	P	t	df	P	t	df	P
Ta (^{0}C)	0.06	129	0.94	0.26	47	0.79	0.26	67	0.79	0.36	40	0.71
T2 (^{0}C)	0.19	129	0.31	0.13	47	0.89	0.31	67	0.75	0.49	40	0.62
T3 (^{0}C)	0.23	129	0.81	0.39	47	0.70	0.37	67	0.71	0.56	40	0.57
SR (W m^{-2})	0.82	129	0.41	0.51	47	0.61	0.38	67	0.70	0.77	40	0.44
wind (m min^{-1})	0.99	129	0.32	0.34	47	0.73	0.50	67	0.61	0.38	40	0.70

all behaviours = mean values for environmental variables, from following individual butterflies, for all behaviour (to give an indication of prevailing conditions in the region during the times of all observations).

flight initiation = mean values for environmental variables recorded at flight initiation.

This indicates that the differences found between the northern and southern populations for wind speed and ground temperature at flight initiation reflect the conditions prevalent in each region.

When the data for flight initiation (from following individual butterflies) are compared with that for flight occurrence (from transect data), for northern butterflies, it appears that although conditions associated with flight initiation reflect the range of conditions "available" to the butterflies, continued flight (inferred from flight occurrence on transects) is subject to a more restricted range of weather conditions. For the northern population, low solar radiation intensity and high wind speed may be limiting factors for active flight for a proportion of the time. For the southern population, solar radiation intensity appears to be less limiting but butterflies also avoid flying in high winds.

7.3.4. Flight duration

Flight duration and environmental variables

Data from following individual butterflies are used to determine the mean and maximum flight duration for each individual. For males from the southern population there is a significant positive correlation between mean flight duration and solar radiation intensity ($r_s = 0.23, P = 0.04, n = 79$), and between maximum flight duration and SR ($r_s = 0.28, P = 0.01, n = 79$). There is a significant negative correlation between mean flight duration and wind speed ($r_s = -0.23, P = 0.04, n = 79$) and between max. flight duration and wind speed ($r_s = -0.30, P = 0.007, n = 79$) (Figure 12). There is no significant correlation between any of the other environmental variables (measures of temperature) and mean flight duration. As there is no co-correlation between solar radiation and wind speed, these can be seen as acting independently on flight duration. The relationship between mean flight duration (s), solar radiation (SR, Wm^{-2}) and wind speed (WS, m min^{-1}) is given by the equation:

mean flight duration = 0.02 SR - 0.31 WS + 24.9 ($r^2 = 0.09, P = 0.02$)

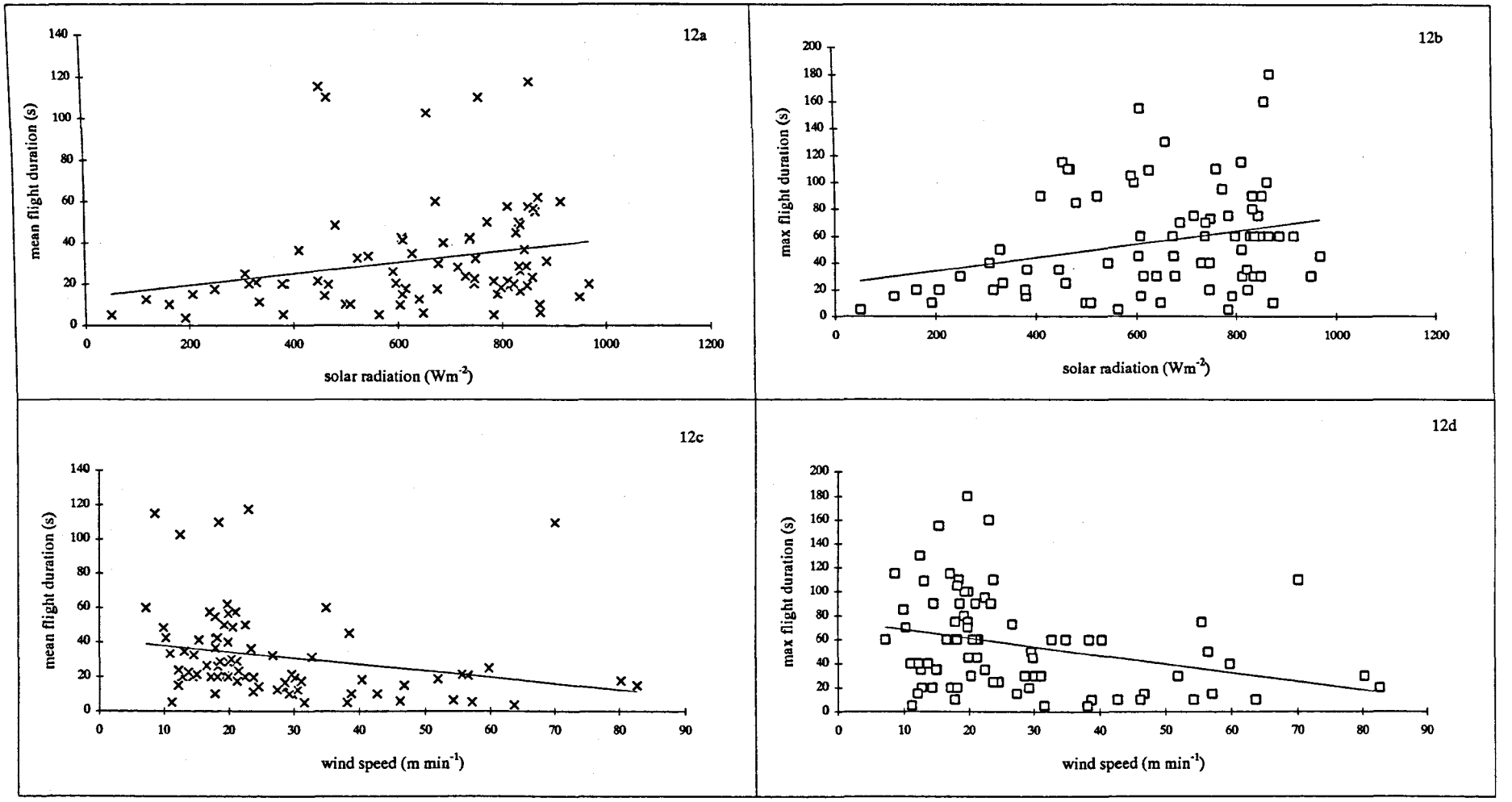


Figure 12. Mean and maximum flight duration in relation to solar radiation intensity and wind speed. Male *M. jurtina,* southern population.

Maximum flight duration is also positively correlated with T_a for southern males (r_s = 0.77, P = 0.03, df = 79). For females from the southern population there is no significant correlation between mean flight duration and any of the environmental variables, although solar radiation approached significance (r^2 = 0.15, P = 0.07, n = 22). Maximum flight duration is positively correlated with SR (r^2 = 0.45, P = 0.03, n = 22), but not with any of the other environmental variables..

In the northern population there is no significant correlation between any of the environmental variables and either mean or max. flight duration for males and females. The largest effect was between T3 (ground temperature) and flight duration for female *M. jurtina* (r^2 = 0.14, P = 0.07, n = 24).

Differences in flight duration between northern and southern populations

Males from the northern population flew for significantly longer than those from the south (mean and maximum flight duration), but there was no difference in flight duration between the females from the two regions. In both regions males flew for significantly longer than females (Table 11, Figure 13).

Table 11. Means (± s.e.) of mean and maximum flight duration (s) in *M. jurtina*, with significance of differences between the sexes and between southern and northern populations (two-tailed t-test, assuming unequal variance).

	n	*mean duration*	*max. duration*
♂ south	79	31.6 ± 2.9	55.9 ± 4.4
♀ south	23	11.9 ± 2.2	25.2 ± 5.4
♂ north	35	111.5 ± 40.3	178.2 ± 46.3
♀ north	24	13.7 ± 2.4	26.1 ±3.9

comparison	*t*	*P*	*t*	*P*
♂ south - ♀ south	3.6	***	3.5	***
♂ north - ♀ north	2.0	*	2.7	**
♂ south - ♂ north	2.9	**	3.9	***
♀ south - ♀ north	0.5	ns	0.1	ns

significance levels: * = 0.05 ≥ P > 0.01, ** = 0.01 ≥ P > 0.001, *** = 0.001 ≥ P
ns = not significant

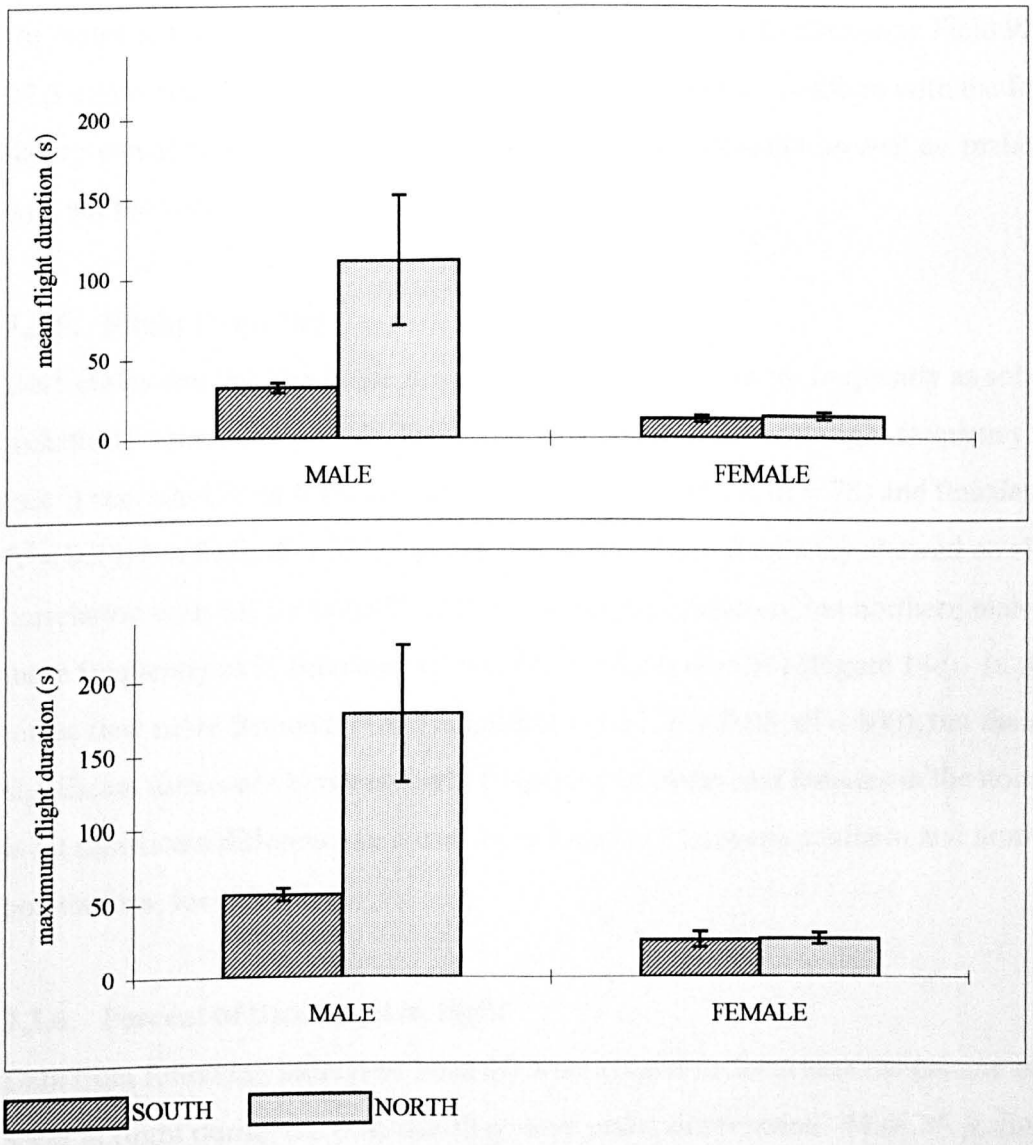


Figure13. Mean and maximum flight duration of *M. jurtina,* southern and northern populations.

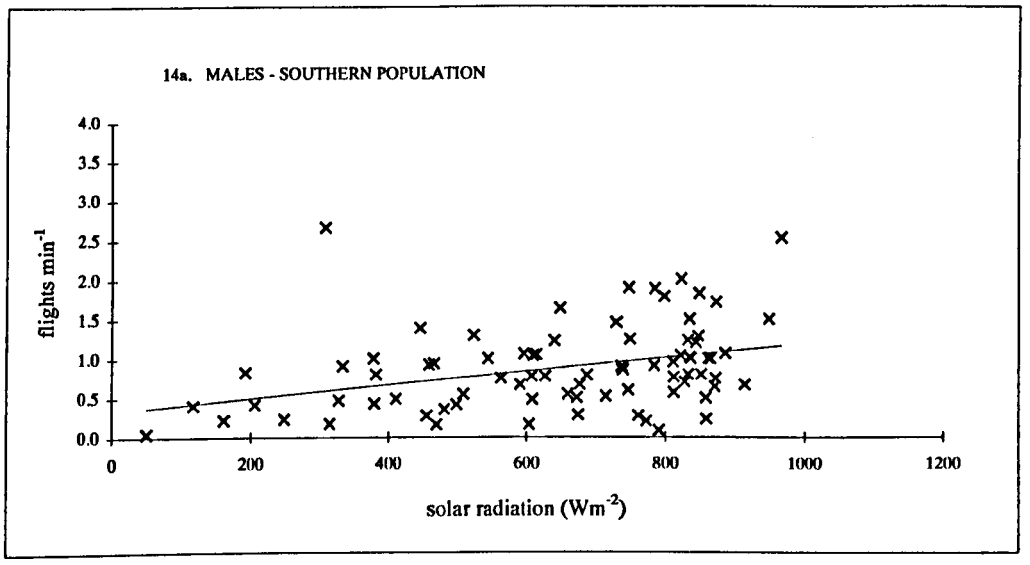
One possibility for the longer flight durations of Scottish males is that many of the flights were carried out to search for suitable nectar sources. Although flower density was not recorded systematically, the general impression was that nectar sources were more abundant on the southern sites. If many of the flights were concerned with foraging for food, rather than females, then one would expect flights of longer duration at sites with fewer or more widely dispersed nectar sources. To test this theory a comparison is made between the two northern sites, Glen Geal (flowers relatively sparse and patchy) and Kinlochaline Field (high density of flowers). The mean flight duration for males at Glen Geal was 118.9 ± 54.8 s (n = 25) and at Kinlochaline Field 92.9 ± 37.5 s (n = 10). This difference is not significant. Another problem with the foraging theory would be that if it were valid it should apply to females as well as males, but this was not the case.

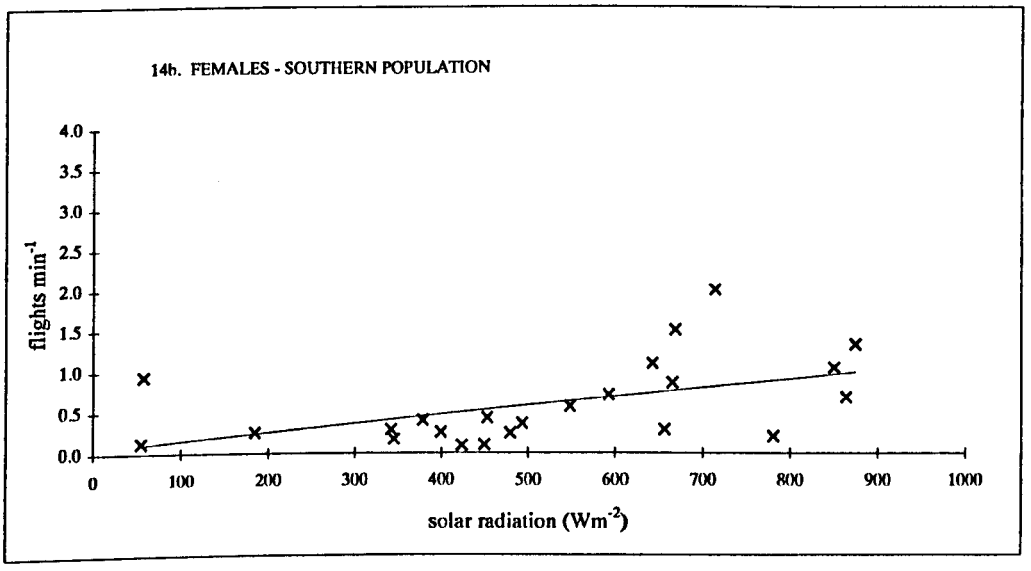
7.3.5. Flight frequency

Both males and females in the southern population flew more frequently as solar radiation intensity increased. The correlation between SR and flight frequency (flights min^{-1}) was significant for both males ($r^2 = 0.12, P = 0.002$, df = 78) and females ($r^2 = 0.24, P = 0.02$, df = 22) (Figures 14a, 14b). Flight frequency showed no significant correlation with SR for butterflies in the northern population, but northern males did fly more frequently as T_a increased ($r^2 = 0.17, P = 0.01$, df = 34) (Figure 14c). In the south males flew more frequently than females ($t = 2.17, P = 0.03$, df = 100), but there is no significant difference between flight frequency of males and females in the north. There is no significant difference in mean flight frequency between southern and northern populations, for either sex.

7.3.6. Percent of time spent in flight

Data from following individual butterflies were used to determine the percent of time spent in flight during the time that they were under observation. Male *M. jurtina* spent a significantly greater proportion of their time in flight than did females, in both northern and southern populations. Scottish males spent a significantly greater proportion of their time in flight than English males, but there was no significant difference for females (Table 12).





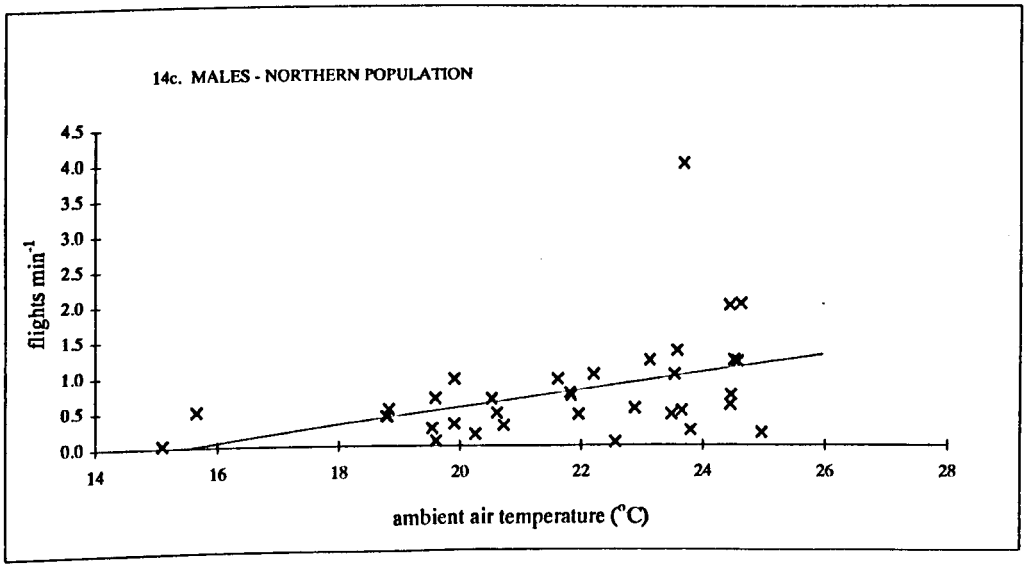


Figure 14. *M. jurtina* flight frequency (flights min^{-1}) in relation to SR (males and females, southern population) and T_a (males, northern population).

Table 12. Mean percent of time spent in flight for male and female *M. jurtina* in southern and northern populations. Mann-Whitney U test used to compare medians.

	n	mean % ± s.e.	comparison	U	*P*
♂ south	79	24.1 ± 3.1	♂ south - ♀ south	311.5	***
♀ south	23	5.4 ± 3.1	♂ north - ♀ north	139	***
♂ north	35	36.4 ± 5.6	♂ south - ♂ north	960	**
♀ north	24	3.8 ± 5.0	♀ south - ♀ north	265	ns

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
ns = not significant

7.4 Discussion

The key results here are associated with flight duration rather than flight initiation. For southern males, there is a positive correlation between mean flight duration and SR and a negative correlation between mean flight duration and wind speed. Maximum flight duration is also positively correlated with SR and T_a, as well as showing a negative correlation with wind speed. Southern females show a positive correlation between SR and max. flight duration, but not between SR and mean flight duration. Mean flight durations represent the general flight activity for the population, whereas maximum flight durations represent the upper limit of activity for the weather conditions (T_a, SR and wind) at that time (Shreeve & Smith 1992).

Shreeve (1984) found a positive correlation between ambient air temperature and maximum (but not mean) flight duration in both male and female *Pararge aegeria* in Bernwood Forest, with males flying for longer than females, especially at high T_a. Shreeve & Smith (1992) found that for two species of speckled wood in Madeira (*P. aegeria* and *P. xiphia*), maximum flight durations were positively correlated with T_a for males and females of *P. aegeria* but for males only in *P. xiphia*. Heinrich (1986a) also found an association between flight duration and T_a for *Coenonympha inornata*. At T_a of less than 18°C all flights were of less than 60 s, but at 29°C most flights lasted for several minutes. Heinrich also suggests that flight duration may be affected by temperatures on the previous day.

The duration of flight will be influenced by whether butterflies are able to remain within the necessary range of T_{th}. Some species show an increase in T_{th} during active flight, e.g. *Danaus plexippus* (Kammer 1970) and *Papilio polyxenes* (Rawlins 1980), but most show a decrease in temperature, e.g. *Coenonympha inornata* (Heinrich 1986a), *P aegeria* (Shreeve 1984) and *Calisto nubila* (Shelly & Ludwig 1985). Heinrich (1983) argues that a decrease in T_{th} during flight is the most usual situation and that convective cooling, hastened by wind, is the main cause. Butterflies in southern and northern populations avoided flying in high winds and for southern males mean flight duration decreased with increasing wind speed. For butterflies like *M. jurtina*, which are not particularly powerful flyers, the effects of high wind could be either mechanical or through their influence on convective cooling. Although butterflies may be able to minimise the effect of wind cooling while they are basking, by choosing sheltered microclimates, once flying wind will lead to cooling and lowering of body temperature (Heinrich 1993).

The fact that most of the correlations between flight duration and weather variables (T_a, SR and wind) applied only to southern males (with exception of max. flight duration and SR for southern females) could be for a number of reasons. The simplest is that this is a statistical effect of sample size - with more than twice as many southern males being included in the analysis than any of the other subsets of butterflies. There are, however, alternative explanations, which are not mutually exclusive. The flight duration of female butterflies was generally much shorter, so it is possible that they were not subject to the same degree of cooling in flight as were males. Northern males generally had the longest flight durations. Different factors affect T_{th} during basking for pre-flight warm-up and during flight, where cooling rate is of greater significance (Heinrich 1983). It is possible that northern males are adapted to decrease their cooling rate during flight by, for example, increased body size to maintain thermal stability (see Chapter 13) or increased furriness to lower convective cooling. Kingsolver & Moffatt (1982) found that *Colias* in cool, windy environments are hairier than those in warm, less exposed sites. Another possibility is that there are enzymatic differences between the northern and southern populations. Goulson (1993) found that ability to sustain prolonged flight

in *M. jurtina* was associated with variation in the phosphoglucose mutase (PGM), an enzyme concerned with metabolic energy production in the flight muscles.

The significantly longer flight duration and percent of time allocated to flight, of males in both regions, compared to females supports the findings of other studies on meadow browns (Pollard 1981, Brakefield 1982a) and other species (Stanton 1982, Kingsolver 1983a). This reflects the different functions of flight in the two sexes, with males patrolling in search of mates, interspersed with visits to nectar plants and females flying to feed and lay eggs.

The importance of patrolling-flight in search of mates may also be a significant factor in the difference in flight duration found between males in the northern and southern populations, with northern males flying for significantly longer than southern males. Meadow browns are described as engaging in both perching and patrolling mate location behaviour (Shreeve 1992), but in this study the high proportion of time spent in flight (especially for northern males) indicates that patrolling was the predominant mate location strategy (see Chapter 8). I suggest that the long flight durations of the northern males are associated with the low population density of *M. jurtina* in the northern site, compared to those in the south (see Chapter 5). If receptive females are in short supply, males would have to fly for much longer to increase their chances of finding one. This hypothesis is consistent with observations on *Colias*, another patrolling species, by Watt *et al.* (1979) who found that dispersal distances of males increased with decreasing population density. Kingsolver (1983b) also notes that *Colias* at mid- and high-elevation sites, with low population density, engage in significantly longer duration flights than those living at higher population density at lower altitudes.

The fact that there was no significant difference between flight duration for females at northern and southern sites again emphasises the different function of flight for the two sexes.

The interaction between weather conditions, flight activity and mate locating strategy is supported by work on species which are territorial, and which perch rather than patrol

while seeking mates. Dreisig (1995) studied territorial male graylings (*Hipparchia semele)* and large skippers (*Ochlodes venata*). These butterflies perch and make flights of short duration in response to passing females or other insects. Dreisig found that there was no increase in flight duration with increasing T_a, but flight frequency increased with increasing ambient temperatures and solar radiation intensity.

Summary

1. Low solar radiation intensities and high wind speed limit flight activity. For southern males, flight duration increases with increasing solar radiation intensity and decreases with increasing wind speed. Butterflies in the northern population may be more restricted in time available for flight, by low solar radiation intensities and high wind speeds, than those in the southern population.

2. Male *M. jurtina* fly for significantly longer duration and spend a greater proportion of their time in flight than females. This is probably due to the different functions of flight in the two sexes.

3. Northern males fly for significantly longer duration and spend a greater proportion of their time in flight than southern males. I suggest that there is a functional requirement for them to do so, as the lower population density means that they must fly for longer to increase their chances of finding receptive females.

Possible adaptations to allow increased flight duration might include larger body size, increased melanization, increased furriness or enzymatic variation (only the first two of which are investigated in this study).

CHAPTER 8. INTERACTIONS WITH OTHER BUTTERFLIES

8.1 Introduction

Here I investigate the interaction of meadow browns with other butterflies (no interactions were observed which involved insects other than butterflies). It is assumed that most of these interactions, which involved short chases, were related to mate locating behaviour. Interactions involving courtship and copulation are described briefly.

The two main strategies used by male butterflies to locate mates are perching (settled males fly to intercept passing females) and patrolling (males fly in search of females) (Scott 1974). Although some species are invariant patrollers, it is recognised that there is a continuum between these two extremes, with many species engaging in both strategies. In *P. aegeria* for example, the tactic adopted is related to aspects of morphology which include hind wing spotting (Shreeve 1987), dorsal wing colour and body size (Van Dyck *et al.* 1997a) and generation (Van Dyck *et al.* 1997b). In other species individual butterflies may switch between perching and patrolling, with the tactic adopted being influenced by temperature and solar radiation (Wickman 1985 1987) density of receptive females (Dennis & Williams 1987) or both (Alcock 1994). Cues for detecting potential mates include movement (used primarily by perching species), colour (used primarily by patrollers) and, in some case, pheromones (Scott 1974, Shreeve 1992). Various theories have been proposed for the evolution of mate-locating behaviour, which include the importance of current ecological circumstances (Ehrlich 1984) and of the ancestral hostplant-habitat structure (Dennis & Shreeve 1988). For a more detailed discussion see Chapter 2, section 2.4.2.

Meadow browns are usually described as being intermediate between patrollers and perchers, with both tactics being found within a single population (Emmet & Heath 1989, Brakefield 1982a, Shreeve 1992) but they are occasionally classified as invariant patrollers (Dennis & Shreeve 1988).

Questions asked in relation to chasing behaviour are:

1. Are chases primarily directed at conspecifics (male or female) or other species, and is there any difference in the duration of chases involving the same or different species?
2. Are chases initiated from perching or patrolling behaviour?

8.2 Methods

Interactions between male meadow browns and individuals of the same or different species (chasing, courtship and mating behaviour) were recorded while walking transects and following individuals. The species involved, the nature and duration of the interaction were all recorded following the protocols described in Chapter 3, section 3.2.3.

8.3 Results

In 1994 (southern population), the majority of interactions observed consisted of male meadow browns chasing another individual. This typically involved a rapid, horizontal flight, with a few twists and turns, with the two individuals in close proximity to each other. These flights usually lasted for 5 - 10 s. Five instances were observed of male and female butterflies engaged in prolonged synchronous flight, rising quite high in the air over Bernwood Meadow. Three instances of non-flight courtship were observed and four copulating pairs were encountered.

In 1995, all interactions observed consisted of males chasing other individuals, with the exception of one copulating pair.

8.3.1 Individuals chased

In 1994 (Southern population) a total of 69 chases were recorded during transects, 64 (92.7%) of which involved another male *M. jurtina*. A total of 62 chases were observed while following individuals, 48 (77.4%) of which involved another male *M. jurtina*.

The mean duration of chases of another male *M. jurtina* was 9 s (range 2 - 60 s, mode 5 s). The mean duration of chases involving other species was 5 s. Only three instances of chases involving female *M. jurtina* were observed (two on transects and one while

following an individual. This last chase lasted 10 s (chases observed on transects were not timed) (Table 13).

Table 13. Species chased by male *M. jurtina*, southern population, with % of total number of chases and mean duration of the chase.

species chased	transect data		data from following individuals		
	n	% chases	n	% chases	mean duration (s)
Maniola jurtina ♂	64	92.7	48	77.4	9
Maniola jurtina ♀	2	2.9	1	1.6	10
Aphantopus hyperantus	1	1.4	6	9.7	6
Melanargia galathea	-	-	2	3.2	5
Pyronia tithonus	1	1.4	-	-	-
Ochlodes venata	-	-	2	3.2	5
Polyommatus icarus	1	1.4	2	3.2	5
Gonepteryx rhamni	-	-	1	1.6	5

In 1995 (northern population) no chases were observed while walking transects. While following individual butterflies, 11 chases were seen, 10 of which involved another *M. jurtina* and the other an *Aglais urticae.*

The small numbers of chases involving individuals of other species makes comparative statistical tests difficult, although it is quite clear that male *M. jurtina* were chased more frequently than either female *M. jurtina* or individuals of other species.

It could be suggested that flying female meadow browns and other species were not as abundant as male *M. jurtina,* and that the percentage of interactions corresponds to their relative overall abundance.

In 1994, *P. tithonus* and *A. hyperantus* were also routinely recorded on transects. Other species, which were chased, were noted as being relatively abundant, although they were not recorded systematically. To test whether individuals of a particular sex or species were being chased disproportionally to their overall abundance, a comparison was made between the relative abundance of that species on the days that chases were recorded (from transect data on the same site on the same day) and the percent of times

that that species was chased. If there was no "selection" of sex or species chased, these two percentages should not be significantly different.

The comparison between relative abundance of a particular sex or species of butterfly (from transect data) and the percent of times that sex or species was chased showed that male *M. jurtina* were being chased in disproportionately high numbers. Female *M. jurtina*, *P. tithonus* and *A. hyperantus* were chased a disproportionately low number of times (Figure 15).

To test whether the "preference" for chasing male meadow browns was statistically significant, a comparison was made between the observed and expected frequencies of species chased. The "preference" for chasing male *M. jurtina* was significant (χ^2 (with Yates' correction) = 106.5, df = 1, P = <0.001) (Table 13a).

Table 13a. Obvserved and expected frequencies of species chased by male *M. jurtina*.

species chased	*observed*	*expected*
male *M. jurtina*	112	57.3
other (female *M. jurtina*, ringlet & gatekeeper)	11	65.6

observed = number of chases involving each species of butterfy (combined data from transects and following individuals).
expected = relative frequency of each species of butterfly encountered on transects x total number of chases (all species).

In 1995 (northern population) other species were not routinely recorded on transects. Other species present at the study sites included *Argynnis aglaja, Boloria selene, Aglais urticae* and *Erebia aethiops*, the latter two species being fairly abundant. It appears that other *M. jurtina* were also being chased in preference to other species (ten chases of *M. jurtina* and one of *A. urticae).* The mean duration of chases of male *M. jurtina* was 8 s (range 1 - 35 s, mode 5 s). The chase of *A. urticae* lasted 2 s.

8.3.2 Behaviour prior to chasing

For each incidence of chasing, the behaviour immediately prior to the chase was noted (the behaviour from which the chase was "launched").

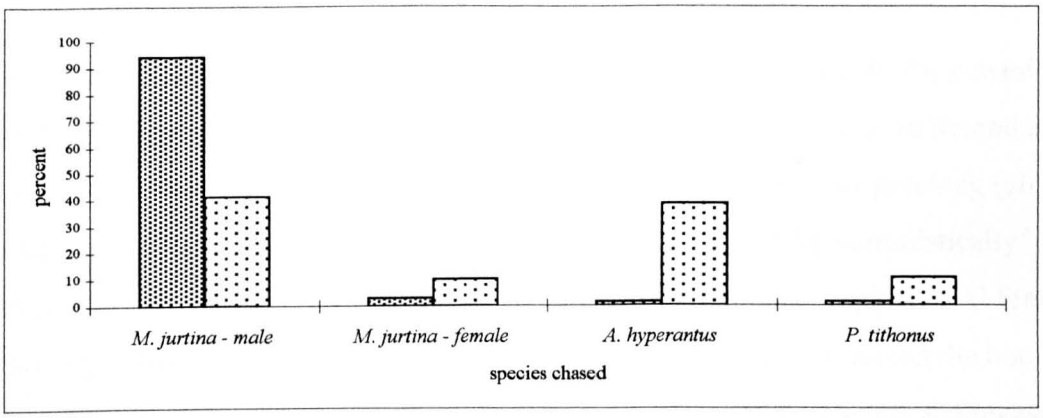


Figure 15. Species chased by male *M. jurtina*, southern population (transect data).
Percent of chases involving a particular species compared to the relative abundance of that species.

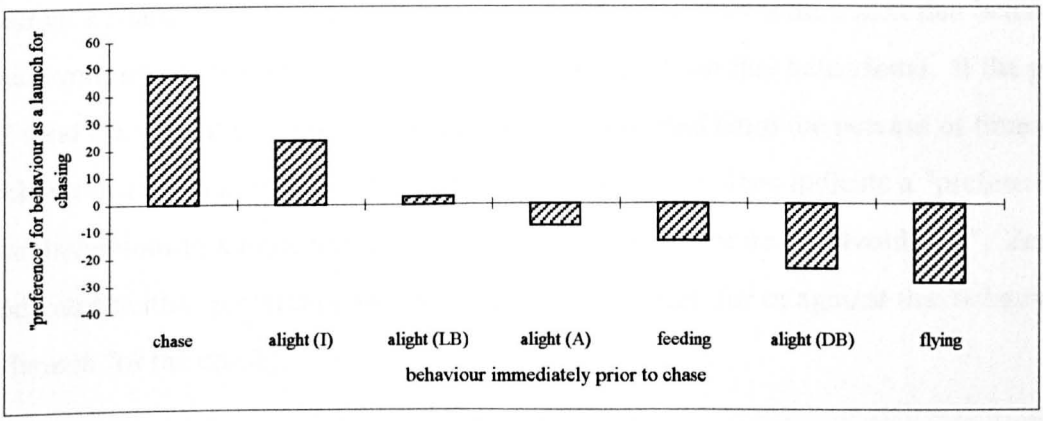


Figure 16. Behaviour immediately prior to a chase. Male *M. jurtina*, southern population.

"Preference" for behaviour as a launch for chasing is derived by calculating the percent of times that behaviour occurs immediately prior to a chase, minus the overall percent of time allocated to that behaviour overall (in individuals which were recorded as chasing).

0 = no preference (behaviour is used as a launch for chasing in the same proportion as the amount of time allocated to it overall).
+ ve values = behaviour is used more frequently as a launch for chasing than would be expected from its overall occurrence.
- ve values = behaviour is used less frequently as a launch for chasing than would be expected from its overall occurrence.

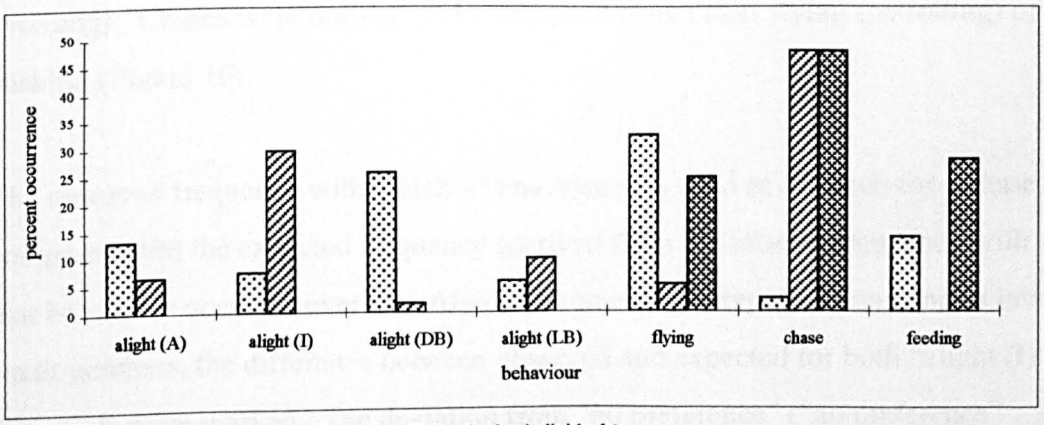


Figure 17. Behaviour immediately before and after chasing. Male *M. jurtina*, southern population.

If the chases occurred exclusively as part of a male's perching or patrolling mate-location behaviour (to investigate the identity of another individual or to defend a perch) it is hypothesised that they would occur primarily with patrolling or perching (alighted) behaviour as the immediate prior activity. If chases occurred "opportunistically" with other activities, such as feeding, being interrupted to investigate any potential females, then any behaviour might occur immediately prior to the chase, provided the butterfly's T_{th} was compatible with active flight (Dennis & Williams 1987, Dennis & Shreeve 1988)

If chases occurred opportunistically, then the relative frequency of a behaviour used to launch a chase should be the same as the relative frequency with which that behaviour occurred overall (i.e. the overall percent of time spent on that behaviour). If the percent of total time spent on a particular behaviour is deducted from the percent of times that behaviour occurs as a launch for a chase, then positive values indicate a "preference" for that behaviour to launch a chase and negative values indicate an "avoidance". Zero indicates neither preference nor avoidance (no selection for or against that behaviour as a launch for the chase).

Data from 1994 (southern population) indicates that the most likely launch for a chase was a previous chase (i.e. if the butterfly was already chasing one individual, there was a good chance that it would switch to chase another individual). Chases were also launched preferentially from alighted (I) (butterfly alighted but neither basking nor heat avoiding). Chases were unlikely to be launched from either flying (patrolling) or dorsal basking (Figure 16).

The observed frequency with which a behaviour was used as a launch for a chase is compared with the expected frequency (derived from the relative frequency with which that behaviour occurred overall). Although some of the expected frequencies involve small numbers, the difference between observed and expected for both "alight (I)" and "chase" is quite marked. The deviation from "no preference" ("no preference" indicating chasing opportunistically) is significant ($\chi^2 = 565.1$, df = 6, $P < 0.001$) (Table 13b).

When the same procedure is applied to chase data for 1995 (Scotland), the observed and expected frequencies are found to be very similar. The small number of observations makes statistical comparisons difficult, but for all behaviours the observed frequency is close to the expected frequency. This indicates that in the northern population chasing was occurring opportunistically (Table 13b).

Table 13b. Observed and expected frequencies with which behaviours were used as a launch for chases.

	SOUTHERN		NORTHERN	
behaviour	*observed*	*expected*	*observed*	*expected*
alight (A)	4	7.96	0	0.03
alight (I)	18	4.52	3	2.72
alight (DB)	1	15.49	1	1.69
alight (LB)	6	3.52	0	0.43
flying	3	19.63	4	4.53
chase	29	1.56	1	0.10
feeding	0	8.32	1	0.48

observed = number of times each behaviour occurred immediatedly prior to a chase.
expected = relative frequency with which each behaviour occurred overall (percent of time allocated to that behaviour by chasing butterflies) x total of "*observed*").

Since data from the southern population indicates that perching was occurring immediately prior to a chase, with butterflies flying up from a perch in the vegetation (at a height of approximately 60 cm) to chase another individual (usually another male *M. jurtina*), the next question is whether these were perches in the same sense as those described for other species, with butterfly returning to the same (or nearby) position once the chase had finished and whether perches were being defended, as in species such as *H. semele*, *O. venata* (Dreisig 1995) and *P. aegeria* (Shreeve 1984, 1987, Van Dyck 1997a, 1997b) with chases being used to see off a potential intruder. To test this hypothesis the data were examined to see what occurred immediately after the chase. If the perches were being used as territories then the butterfly would be expected to return to the perch either immediately after the chase or following a short flight.

Of the 29 chases launched from when the butterfly was alighted, there were no cases of the butterfly returning and perching again. In all cases of chasing (whatever the previous behaviour had been), the butterflies either went on to chase another individual,

landed on a flower and began feeding or continued flying with a typical patrolling flight (Figure 17).

8.3.3 Prolonged male - female interactions

In July 1994 (southern population), on five occasions, male and female *M. jurtina* were observed in prolonged ascending flight over Bernwood Meadow. The flight was fairly slow, and of quite a different character to the short chase flights. This behaviour could be interpreted as flight to escape from a male by an unreceptive female, similar to that described in other species (Shapiro 1970, Wiklund & Ahrberg 1978, Wiklund 1982).

On three occasions in 1994 non-flight courtship was observed, with male butterflies walking around, rubbing and fluttering their wings over a sedentary female. All these courtships occurred low down (< 10 cm) in the grass.

On four occasions in 1994 copulating pairs of *M. jurtina* were observed. Two of these were low in the grass (≅ 10 cm). One was on a leaf at the edge of a ride (≅ 20 cm) and one was ≅ 3 m high in a tree.

In 1995 (Scotland) only one occasion of prolonged male - female interaction was observed. This was a pair of *M. jurtina* copulating, low in the grass.

All observations of copulating pairs were made on transects. Pairing was prolonged, with butterflies still present in the same place up to 63 minutes later.

8.4 Discussion

Species chased

The most frequently recorded encounters were of male meadow browns chasing another male meadow brown. These encounters were also of longer duration than those directed at other species of butterfly. Ravenscroft (1994) found that 69% of flights elicited by other insects, in male chequered skippers (*Carterocephalus palaemon* - a territorial percher) were directed at hetero- rather than conspecifics, mainly bees and other butterflies. These interactions were, however, briefer than those directed at either males

or females of the same species (mean of 2.8 s compared to 9.1 s and 12 s). Dreisig (1995) also found that in territorial male graylings most encounters were directed at other butterfly species (32%) compared to 12% for male conspecifics and 2% for female conspecifics. Again, the mean duration of encounters with conspecifics was longer than those with heterospecifics (24 s and 5.5 s respectively). Unlike these last two examples, the frequency of encounters in the large skipper showed a pattern more similar to that described here for the meadow brown, with conspecific encounters accounting for 52% and other butterfly species 17%. The mean duration of conspecific encounters was again longer than that with heterospecifics (17.5 s and 6.6 s respectively).

Visual cues such as movement, size and colour are the most likely signals to elicit an initial response by male *M. jurtina* (Silberglied 1994). If this is the case, and if the assumption is made that these chases are "inspection flights" (Brakefield 1982a, Dreisig 1995) to determine the species and sex of the individual at close range, then the longer duration of encounters with conspecific males, compared to heterospecifics, fits these assumptions. Presumably it takes longer to distinguish a male of the same species from a female, than it does to recognise that a different species is involved.

However, this does not explain why male *M. jurtina* were chased in disproportionately high numbers, compared to their overall occurrence. One explanation might be that the *apparent* density of males is much higher than that of females (even if actual densities are similar), because of the high proportion of time males spend in flight compared to females.

Behaviour before and after chasing

In the southern population chases were launched in disproportionately high numbers from either a previous chase or from an alighted (intermediate) posture. The high incidence of one chase leading to another could be due to the increased visual apparency of the pair of butterflies. If size and movement elicits a response, then the chasing pair could attract more attention than an individual butterfly, and so lead to a third individual coming to investigate.

The disproportionately high number of chases launched from alighted behaviour seems to confirm that *M. jurtina* are using perching as a mate location strategy. However, as Dennis & Shreeve (1988) comment, it is sometimes difficult to distinguish perching (a particular location being used for the express purpose of locating mates) from other behaviours such as resting or thermoregulatory activity (although these activities are not necessarily mutually exclusive). The fact that southern males tended to roost at a greater height in the vegetation than females suggests that they may be using perches with a better visibility to increase the chances of locating a female. It is clear that *M. jurtina* were not behaving in a territorial manner, as there was no evidence of perches being defended or even returned to after a chase.

There was also no evidence that the relative frequency of the two tactics of perching and patrolling was influenced by either time of day or weather conditions, as has been found for other species (Wickman 1985, 1987, Dennis & Williams 1987, Alcock 1994).

One rather surprising find was that, given the proportion of time that males spent in patrolling flights (flying slowly, low over the top of the vegetation), so few chases were observed during the course of these flights, compared to the number of chases which were launched by alighted butterflies. One explanation for this could be that during patrolling flights males are scanning the ground and vegetation in search of alighted females, so pay less attention to airborne insects. In contrast, alighted males have an "eye to the sky" so that airborne butterflies passing within their range of vision are more likely to elicit a response.

In the northern population interactions with another individual occurred opportunistically, with chases likely to be launched from any activity, in a similar way to that described for one of the mate location strategies of *O. venata* (Dennis & Williams 1987).

The opportunistic nature of mate location in the northern population does not conform to the models proposed by Scott (1974) and Ehrlich (1984) which predict that perching is more likely to evolve in low density populations where the energetic costs of

patrolling, in search of scarce females, are high. Although the small sample size of chases in the northern population makes some comparisons difficult, of the eleven chases observed, five (45%) were initiated by flying males, compared to only three (5%) in the southern population. Northern males spent a high proportion of their time patrolling and this seemed to be the predominant mate locating strategy for this population, together with opportunistic investigation flights while engaged in other behaviours. The lower height in the vegetation at which northern males alighted (0 - 5 cm), compared to southern males, also supports the view that they were not using perches as vantage points with good visibility.

I suggest that two factors may be relevant here. The first is that although the population is at low density, the habitat patches used by northern meadow browns in this study were small and isolated. This means that by patrolling, the males could cover a large proportion of the habitat where receptive females were likely to be found in a relatively short time. By crossing and re-crossing the same area repeatedly, they would increase their chances of finding newly eclosed receptive females. Locating alighted females may possibly be easier for northern males, as there is a tendency for increased apparency towards the north, with individuals having a larger fulvous band on the forewing, with increased size of both dorsal and ventral apical eyespots, with increased contrast and brightness (Dennis & Shreeve 1989). If they had adopted a sit and wait perching strategy, the very low density of airborne females means that the chances of one flying within visual range would be small. At the same time, it would obviously be advantageous to investigate any potential mates which were encountered during the course of other activities (basking, feeding etc.).

CHAPTER 9. ALIGHTED BEHAVIOUR: BASKING, HEAT AVOIDING AND INTERMEDIATE POSTURE

9.1 Introduction

Butterflies regulate their body temperature through adjustment of posture and orientation in relation to the sun (Kingsolver 1985a, Shreeve 1992, Dennis 1993) and by choice of microclimate, substrate and height (Casey 1981, Shreeve 1984, Rutowski *et al.* 1994). Adjustments take place in the context of body temperature, ambient air temperature (T_a) and solar radiation (SR) intensity. These will in turn be influenced by time of day, weather and geographic location. The posture adopted will also influence the apparency of the butterfly to conspecifics and predators, depending on whether the dorsal or ventral wing surfaces are exposed. Postures adopted by resting but potentially active butterflies are conservative within taxonomic groups (Shreeve & Dennis 1992) and not usually subject to proximal, short term influences of habitat and climate (e.g. *Coenonympha pamphilus*, a lateral basker, always uses the wings-closed posture throughout its range). *M. jurtina* is usually described as a dorsally basking species (Emmet & Heath 1989, Thomas & Lewington 1991, Shreeve 1992).

A number of specific questions are asked here in relation to alighted behaviour of *M. jurtina*:

1. Does time of day influence basking posture?
2. Are the different postures adopted associated with differences in T_a and SR intensity? (i.e. it is predicted that a heat avoiding posture would be associated with higher T_a and SR intensity, and a basking posture with lower T_a and SR intensity).
3. Are there any differences between males and females and between butterflies in southern and northern regions in the postures adopted at specific T_a and SR intensity?
4. Are there any differences between males and females and between butterflies in southern and northern regions in the duration of each category of alighted behaviour?
5. Is there a relationship between T_a and SR levels and duration of basking, heat avoiding or intermediate posture?
6. At what heights are butterflies alighted and are there any differences between the sexes or between the regions in the heights adopted?

7. Do butterflies select different height substrates to settle on at different times of day?
8. Which substrates are used by the alighted butterflies?

9.2 Methods

The term "alighted" is used here to describe butterflies, which were "resting", but potentially active and not engaged in other activities such as feeding or egg laying. The data used for analysis of alighted behaviour were obtained from following individual butterflies. The following variables were recorded for alighted butterflies: height, substrate, orientation, wing angle (0° = wings closed, 180° = wings fully open), time and duration. Environmental variables were recorded simultaneously for all behavioural observations (see Chapter 3, section 3.2).

The behaviour of alighted butterflies is classified into four categories, depending on wing angle and orientation in relation to the sun:

dorsal basking (DB) - wings open (angle between the wings ≥ 140°), butterfly orientated towards the sun (body axis at 0° to solar radiation)

lateral basking (LB) - wings closed, butterfly orientated with wings perpendicular to the sun (body axis 90° to solar radiation).

heat avoiding (A) - wings closed, butterfly orientated with wings parallel to solar radiation (body axis at 0° to solar radiation).

intermediate posture (I) - wing angle and/or body orientation intermediate between the above categories.

9.3 Results

9.3.1 Time of day and basking posture.

Although meadow browns are normally described as dorsal baskers, if the wings were closed, orientated at right angles to incoming solar radiation (in some cases tilted to maximise the wing surface area exposed), then this is interpreted as lateral basking. Lateral basking was observed in both male and female butterflies in southern and northern regions. Lateral basking accounted for 37% of all basking episodes observed, with individual butterflies using both dorsal and lateral postures. Lateral basking

usually took place with the wings in an upright (vertical) position, but tilting (to maximise the surface area exposed to direct solar radiation) was observed in 22% of cases, with butterflies tilted at angles of between 30° and 60° from the vertical position. One influence on the posture adopted by an individual might be the angle and position of the sun (e.g. overhead, south, at around midday*; low, east in the morning; low, west in the late afternoon). To investigate this, the percent of dorsal or lateral basking butterflies at different times of day was determined. Between 08.00 and 10.00 all basking male butterflies, of both northern and southern populations, adopted the lateral posture. The percent of laterally basking male butterflies decreased towards midday/early afternoon (percent of dorsal basking increasing) as the sun moved higher, and then increased again towards late afternoon (Figure 18). There are less data available for female butterflies and the pattern is not so clear cut, but there appears to be an increase in lateral basking (decrease in dorsal basking) around midday/early afternoon - i.e. the opposite pattern to the males.

It can be argued that solar radiation level rather than angle of the sun is the main influence on basking posture, with the high percent of laterally basking male butterflies occurring in the morning because solar radiation levels were lower - rather than because the sun was at a lower angle in the sky. This hypothesis is tested using the group for which most data are available - males from the southern population. Data are grouped into butterflies basking between 08.00 and 12.00 with solar radiation levels greater than 500 Wm^{-2} , and butterflies basking between 12.00 and 16.00, with solar radiation levels less than 500 Wm^{-2}. If solar radiation level, rather than sun angle, is the main influence on basking posture, then butterflies in the low solar radiation group should show a higher percent of lateral basking than those in the high solar radiation group. This is not the case: male *M. jurtina* basking in the morning, under conditions of high solar radiation, still show a higher percent of lateral basking (Figure 19), suggesting that for male butterflies sun angle may be more important than absolute levels of solar radiation in influencing basking posture.

* All times were recorded as BST (one hour later than GMT), so the sun was at its zenith at 13.00.

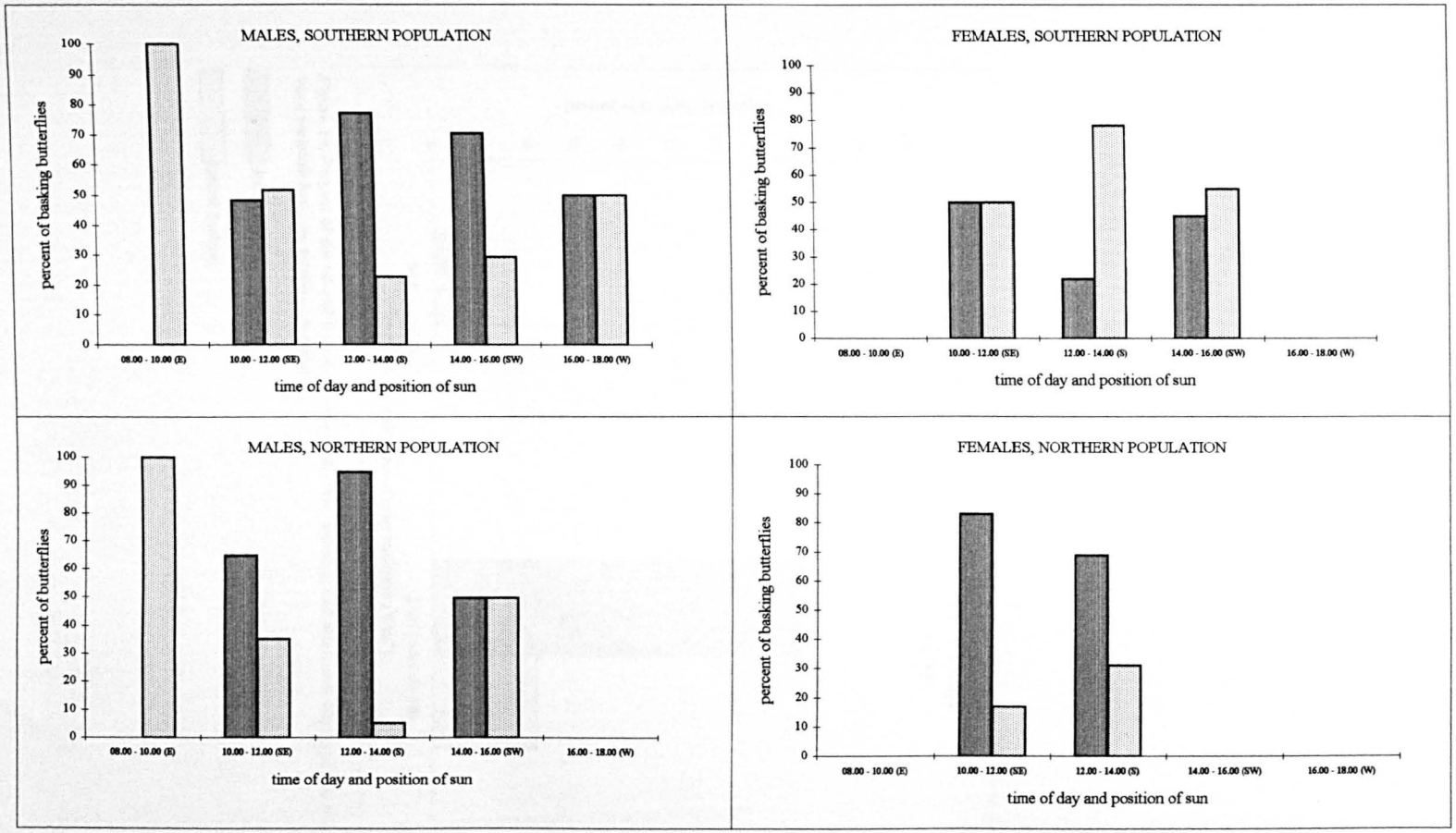


Figure 18. Basking behaviour at different times of day and sun position

dorsal basking

lateral basking

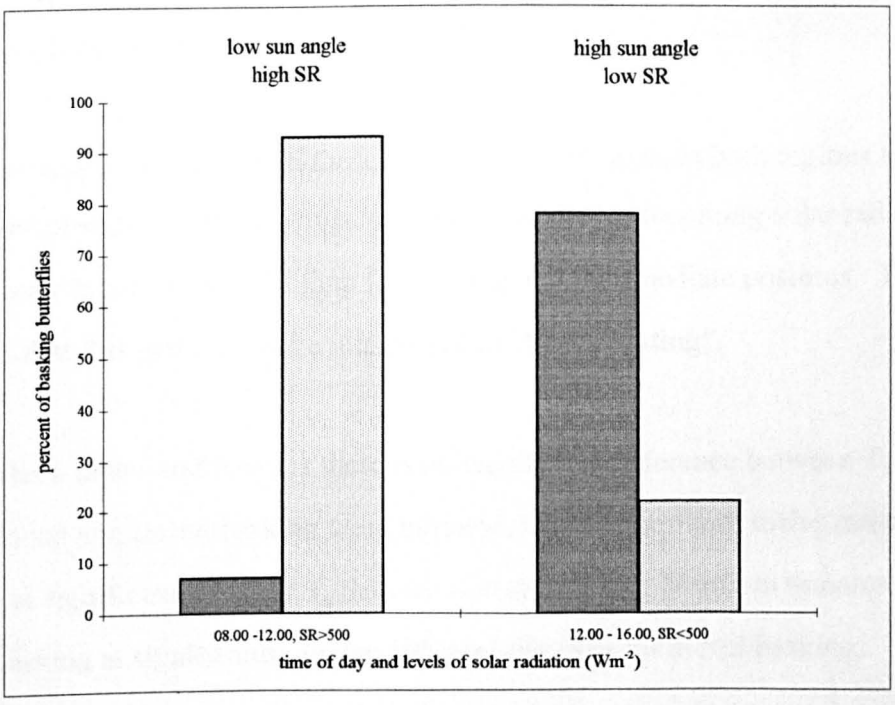


Figure 19. Percent of dorsal and lateral basking butterflies, morning and afternoon, high and low levels of solar radiation. Male meadow browns, southern population.

dorsal basking

lateral basking

9.3.2 Ambient air temperatures and solar radiation intensity associated with initiation of basking, heat avoiding and intermediate postures.

For each individual followed, values for T_a and SR at the initiation of; basking, heat avoiding or intermediate posture were recorded. These were then used to determine population means for male and female butterflies in the southern and northern populations. In analyses involving initiation of alighted behaviour, T_a is used in preference to ground temperature as butterflies were usually flying immediately prior to alighting, so this is assumed to give a better approximation of the temperature which they were experiencing.

Table 14 shows the summary data for T_a and SR intensity at which the different categories of alighted posture were initiated, together with the significance of differences between the postures.

The main trend to emerge from these data is that both sexes in both regions tended to initiate the posture of closed wings, orientated parallel to incoming solar radiation, at significantly higher T_a and SR than for basking and intermediate postures. This confirms that this posture can be interpreted as "heat avoiding".

For southern males and females there is no significant difference between T_a and SR at which dorsal and lateral basking were initiated, whereas northern males initiated lateral basking at significantly higher T_a than for dorsal basking. Northern females initiated lateral basking at significantly higher SR intensity than for dorsal basking.

There are no clear cut trends for differences between basking and intermediate posture: There is no significant difference between the two for southern males or for northern females, whereas southern females initiate basking at lower T_a but higher SR than for intermediate posture. Northern males also initiate basking at higher SR intensity than for intermediate posture (Table 14).

Table 14. Mean T_a and SR (± S.E) associated with initiation of alighted behaviour, with significance of differences between categories of alighted behaviour. (two-tailed t-tests, assuming unequal variance).

		MALES			FEMALES				MALES		FEMALES	
	ACTIVITY	N	T_a (°C)	SR ($W m^{-2}$)	N	T_a (°C)	SR ($W m^{-2}$)	COMPARISON	T_a	SR	T_a	SR
SOUTH	DB	72	20.5 ± 0.3	591.5 ± 24.3	25	18.0 ± 0.5	548.1 ± 32.7	DB - LB	NS	NS	NS	NS
	LB	43	21.5 ± 0.4	629.9 ± 30.9	37	19.2 ± 0.4	566.2 ± 30.0	B - I	NS	NS	I > B t = 3.05 **	B > I t = 2.41 *
	B	115	20.9 ± 0.2	605.8 ± 19.1	62	18.7 ± 0.3	558.9 ± 22.2	B - A	A > B t = 4.66 ***	A > B t = 3.93 ***	A > B t = 3.99 **	A > B t = 2.06 *
	I	86	21.3 ± 0.2	663.3 ± 26.7	61	20.1 ± 0.3	466.0 ± 29.2	I - A	A > B t = 4.36 ***	A > B t = 2.13 *	A > B t = 2.14 *	A > B t = 3.50 ***
	A	45	23.0 ± 0.3	739.9 ± 24.0	21	21.7 ± 0.7	662.5 ± 48.0					
NORTH	DB	40	20.4 ± 0.3	504.1 ± 36.9	37	18.9 ± 0.2	316.8 ± 27.1	DB - LB	DB > LB t = 2.17 *	NS	NS	LB > DB t = 3.93 **
	LB	14	21.6 ± 0.4	440.2 ± 50.9	11	20.4 ± 0.8	653.8 ± 81.2	B - I	NS	I > B t = 2.75 **	NS	NS
	B	54	20.7 ± 0.2	487.6 ± 30.4	48	19.2 ± 0.3	394.1 ± 34.4	B - A	A > B t = 5.82 **	NS	A > B t = 6.39 ***	A > B t = 7.58 ***
	I	64	20.4 ± 0.2	369.5 ± 30.3	90	19.2 ± 0.2	411.6 ± 28.8	I - A	A > B t = 6.64 ***	A > B t = 2.78 *	A > B t = 6.56 ***	A > B t = 7.84 ***
	A	4	23.4 ± 0.4	510.9 ± 40.9	20	21.9 ± 0.3	760.2 ± 33.8					

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant.
DB = dorsal basking, LB = lateral basking, B = basking (dorsal & lateral), I = intermediate posture, A = heat avoiding.

Ground hugging

Ground hugging (wings appressed down on the substrate - included here with dorsal basking) was observed in 9 dorsally basking southern males (12% of dorsal basking records) and 1 northern female. When the mean T_a at which ground hugging occurred is compared to the mean T_a for the non-ground hugging dorsal basking, there is no significant difference. Ground hugging did, however, occur at significantly lower SR intensity (456.8 ± 35.4 Wm^{-2} for ground hugging, compared to 612.3 ± 27.1 Wm^{-2} for non-ground hugging , $t = 3.49, P = 0.002$, df = 19).

9.3.2.1 Summary of differences between T_a and SR associated with initiation of the different alighted postures.

The main picture to emerge is that, as expected, heat avoiding was initiated at higher T_a and SR levels than basking or intermediate posture. There is no consistent difference between T_a and SR levels for lateral and dorsal basking or between basking and intermediate posture.

9.3.3 Differences between sexes and regions in mean T_a and SR at initiation of basking, heat avoiding and intermediate posture.

Table 15 shows the differences between mean ambient air temperature and solar radiation intensity at initiation of alighted behaviour: between southern and northern regions, and between sexes within each region.

Basking

Females in southern and northern regions initiated basking behaviour at lower T_a and SR levels than males. The difference in T_a is significant for both regions. The difference in SR is significant in the northern region but not in the south. There is no significant difference between the regions in the T_a at which basking was initiated, for either males or females. Males and females in the northern population initiated basking at lower SR levels than males and females in the southern population. The difference is significant for both males and females (Table 15).

Table 15. Differences between mean ambient air temperature (T_a, °C) and solar radiation intensity (SR, Wm^{-2}) at initiation of alighted behaviour: between southern and northern regions, and between sexes within each region. (two-tailed t-test, assuming unequal variance).

	ACTIVITY					
	BASKING		*INTERMEDIATE POSTURE*		*HEAT AVOIDING*	
COMPARISON	T_a	*SR*	T_a	*SR*	T_a	*SR*
southern ♂ *southern* ♀	♂ > ♀ t = 5.31 ***	NS	♂ > ♀ t = 3.09 **	♂ > ♀ t = 4.99 ***	NS	NS
northern ♂ *northern* ♀	♂ > ♀ t = 3.99 ***	♂ > ♀ t = 2.04 *	♂ > ♀ t = 3.09 **	NS	♂ > ♀ t = 2.93 *	♀ > ♂ 4.69 **
southern ♂ *northern* ♂	NS	south>north t = 3.30 ***	south>north t = 2.69 **	south>north t = 7.28 ***	NS	south>north t = 4.82 **
southern ♀ *northern* ♀	NS	south>north t = 4.03 ***	south>north t = 2.39 *	NS	NS	NS

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant

Intermediate posture

Females in the southern and northern regions initiated intermediate posture at significantly lower T_a than males. In the south the difference in SR between males and females (♀ < ♂) is also significant. Males in the northern region initiated intermediate posture at significantly lower T_a and SR levels than those in the south. Females in the north also initiated intermediate posture at significantly lower T_a than those in the south, but there is no significant difference between SR levels for females in the north and south (Table 15).

Heat avoiding

There are no clear trends between the sexes or the regions for heat avoiding behaviour. In the south there is no significant difference between males and females for T_a or SR. In the north, females initiate heat avoiding behaviour at lower T_a but higher SR than males. Between the regions, females show no significant difference, but males initiate heat avoiding at higher temperatures in the south than in the north (Table 15).

9.3.3.1 Summary of differences between sexes and regions in mean T_a and SR at initiation of basking, heat avoiding and intermediate posture.

Males and females

These data show that in the southern region females appear to operate at lower T_a and SR intensity than males, initiating basking, intermediate postures and heat avoiding at lower T_a and SR than males. In the north the picture is the same for T_a, with females initiating basking, intermediate postures and heat avoiding at lower T_a than males. For solar radiation the difference between males and females in the north is less clear cut; basking by females was initiated at a slightly lower SR intensity than males, but heat avoiding at higher SR intensity.

North and south

Males in the north initiated basking, intermediate postures and heat avoiding at lower levels of solar radiation than those in the south. The main difference between females in the north and south is in the lower solar radiation intensity at which northern females initiated basking. Males and females in the north both initiated intermediate postures at lower ambient temperatures than those in the south, but there is no significant difference between the regions in the temperatures at which basking and heat avoiding were initiated.

The overall tendency is for females to operate at lower T_a and SR levels than males, and for males in the north to operate at lower T_a and SR levels than those in the south.

9.3.4 Differences between sexes and regions in mean duration of basking, heat avoiding and intermediate posture.

For each individual followed values for duration of basking, heat avoiding and intermediate posture were determined. These were then used to give population means for male and female butterflies in the southern and northern populations. In a few cases (usually in very poor weather conditions) bouts of alighted behaviour lasted for extended periods of time (more than an hour). Reference to field notes suggested that these butterflies were roosting - i.e. not potentially active, so these data points were removed from the analysis.

Differences between males and females

In the south there was a tendency for females to adopt basking, intermediate, and heat avoiding postures for slightly longer duration than males, but none of the differences in duration are significant. In the north the mean duration for dorsal basking is significantly longer for females than for males, but there is no significant difference in the duration of lateral basking. Females in the north also engaged in heat avoiding for significantly longer duration than males (Table 16).

Table 16. Duration (s) of alighted behaviour (mean ± s.e.) with significance of differences: between southern and northern regions, and between sexes within each region. (two-tailed t-test, assuming unequal variance).

		MALES		*FEMALES*	
	ACTIVITY	*N*	*MEAN ± S.E.*	*N*	*MEAN ± S.E.*
SOUTHERN	*dorsal basking*	72	90.7 ± 31.5	25	82.6 ± 21.7
	lateral basking	43	72.6 ± 13.9	37	128.6 ± 44.3
	intermediate	85	69.8 ± 24.7	61	118.7 ± 25.2
	heat avoiding	45	90.8 ± 23.9	21	146.3 ± 42.3
NORTHERN	*dorsal basking*	39	69.3 ± 18.4	37	289.2 ± 70.3
	lateral basking	14	106.7 ± 26.7	11	84.9 ± 26.8
	intermediate	64	264.6 ± 59.3	90	216.7 ± 60.9
	heat avoiding	4	32.7 ± 8.7	20	177.0 ± 55.2

	ACTIVITY			
COMPARISON	*dorsal basking (DB)*	*lateral basking (LB)*	*intermediate (I)*	*heat avoiding (A)*
southern ♂ - *southern* ♀	NS	NS	NS	NS
northern ♂ - *northern* ♀	♀ > ♂ t = 3.02 **	NS	NS	♀ > ♂ t = 2.58 *
southern ♂ - *northern* ♂	NS	NS	north > south t = 3.03 **	south > north t = 2.28 *
southern ♀ - *northern* ♀	north > south t = 2.81 **	NS	NS	NS

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant

Differences between north and south

There is no significant difference between the mean duration of either dorsal or lateral basking between males in the north and south. Males in the north adopted intermediate postures for significantly longer than those in the south, but spent significantly less time heat avoiding. The mean duration of dorsal basking is significantly longer for females in the north than for females in the south, but there is no significant difference in the duration of lateral basking. There is no significant difference between females in the north and south in the mean duration of intermediate posture or heat avoiding (Table 16).

9.3.4.1 Summary of differences in duration of alighted postures

The main feature to emerge from these data is the long duration of dorsal basking by females in the north. The mean duration of each episode of dorsal basking by northern females was nearly 5 minutes - significantly longer than for either northern males (≅ 1 minute) or southern females (≅ 1 min 20 sec). There is no difference between mean basking duration for males in the north and south.

9.3.5 Relationship between T_a, SR, ground temperature (T3) and duration of basking, heat avoiding and intermediate posture.

Regression analyses were carried out to test whether there was a relationship between T_a or SR and duration of any of the alighted categories of behaviour. All significant relationships were found to be curvilinear, so duration data were log transformed to give linear regression results. As butterflies were often alighted on or near the ground, ground temperature (T3) was included in the analysis as a possible influence on duration of behaviour.

There is no significant relationship between T_a and duration of any of the alighted categories of behaviour.

There is a significant negative regression between duration of lateral basking and both SR and ground temperature (T3) for males in the southern population (i.e. duration of

basking decreased as SR and T3 increased), but no significant relationship between duration of dorsal basking and either SR or T3 (Table 17, Figure 20)

For males in the northern population there is a significant negative regression between duration of dorsal basking and both SR and ground temperature (T3), but no significant relationship between duration of lateral basking and either SR or T3 (Table 17, Figure 21). For females in the north and south there is no significant relationship between basking duration and either SR or T3.

Females in the south and in the north both show a significant negative relationship between duration of intermediate posture and SR, but no relationship between T3 and duration of intermediate posture (Table 17, Figure 22).

Duration of heat avoiding showed no relationship with either SR or T3 for males and females in both regions.

9.3.5.1 Summary of influence of environmental variables on duration of alighted behaviour.

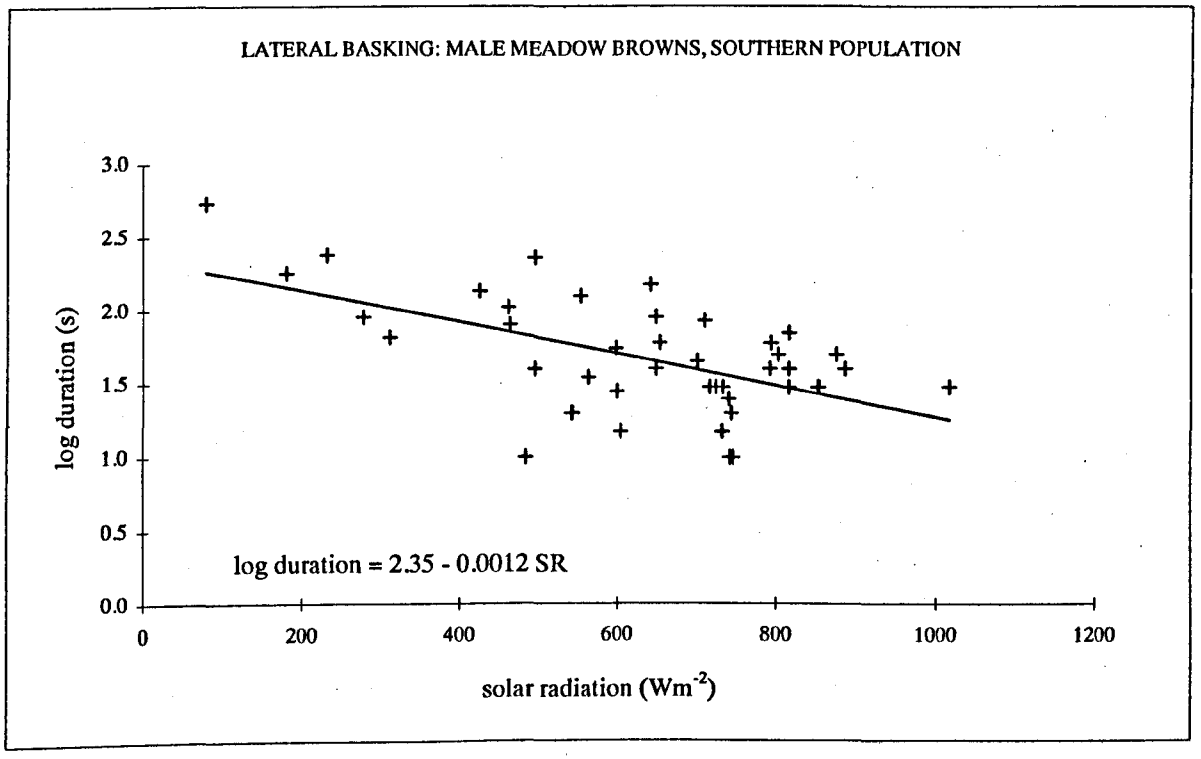
It was predicted that duration of basking should decrease as temperature and solar radiation levels increase. This is confirmed for solar radiation levels and basking duration for males in both south (lateral basking) and north (dorsal basking), but not for females. The similar pattern shown for T3 and basking behaviour may be spurious, as SR and T3 are strongly correlated (see Chapter 4, section 4.2.1). To test whether this is the case, the residuals of the regression between SR and mean log duration were regressed against T3. There is no significant relationship, which suggests that T3 has no further independent influence on duration of basking behaviour.

It was predicted that duration of heat avoiding should increase as SR and temperature increased. This prediction is not confirmed. No predictions were made about the duration of intermediate posture and environmental variables. Females in both populations showed a pattern for intermediate posture similar to that for basking in males - i.e. as solar radiation increased, the duration of intermediate posture decreased.

Table 17. Regression of $\log_{10}$ duration of alighted behaviour on solar radiation (SR, Wm^{-2}) and ground temperature (T3, °C).

	dorsal basking		*lateral basking*		*intermediate posture*		*heat avoiding*	
	SR	*T3*	*SR*	*T3*	*SR*	*T3*	*SR*	*T3*
♂ *south*	NS	NS	$r^2 = 0.32$ df = 42 ***	$r^2 = 0.14$ df = 42 **	NS	NS	NS	NS
♀ *south*	NS	NS	NS	NS	$r^2 = 0.09$ df = 60 *	NS	NS	NS
♂ *north*	$r^2 = 0.19$ df = 39 **	$r^2 = 0.41$ df = 39 ***	NS	NS	NS	NS	-	-
♀ *north*	NS	NS	NS	NS	$r^2 = 0.14$ df = 89 ***	NS	NS	NS

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant.
(All regressions are negative).



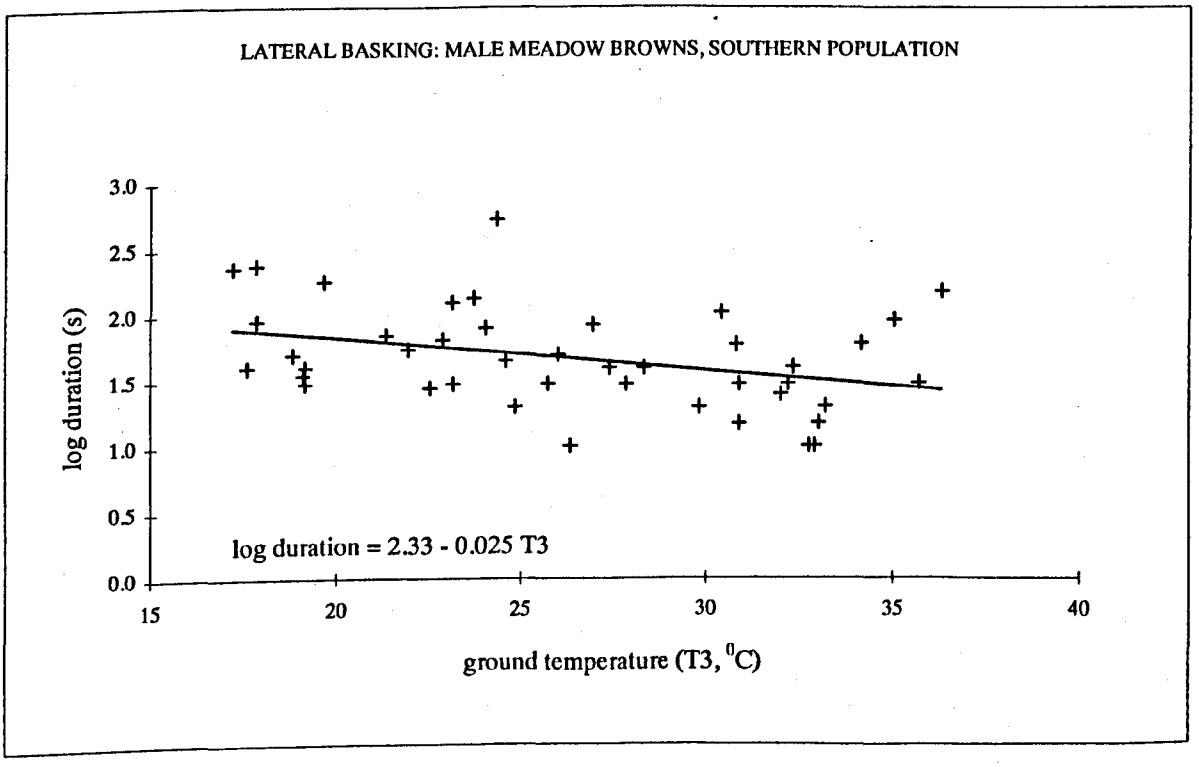


Figure 20. Duration of lateral basking at different solar radiation intensities and ground temperatures. Male *M. jurtina*, southern population.

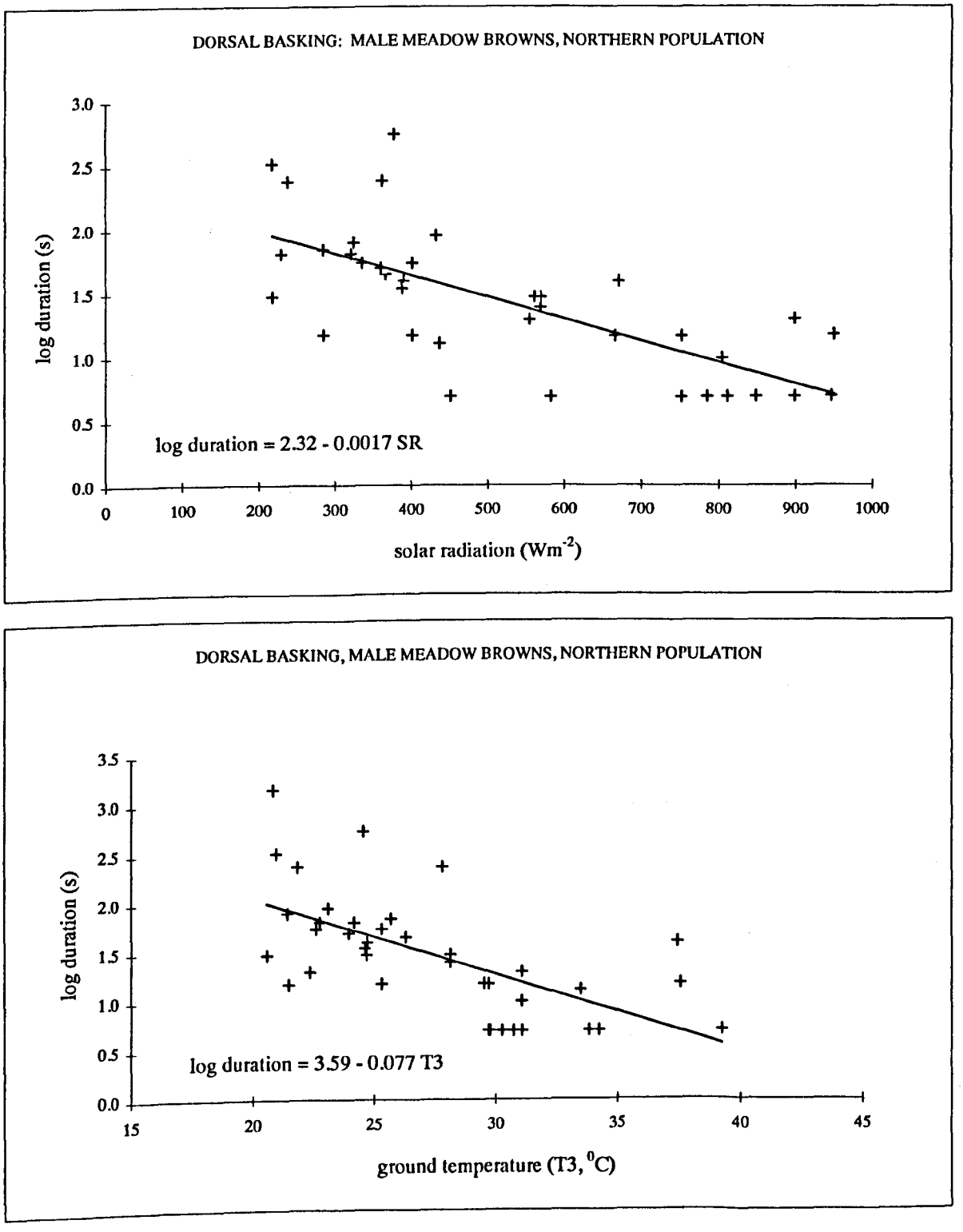


Figure 21. Duration of dorsal basking at different solar radiation intensities and ground temperatures. Male *M. jurtina*, northern population.

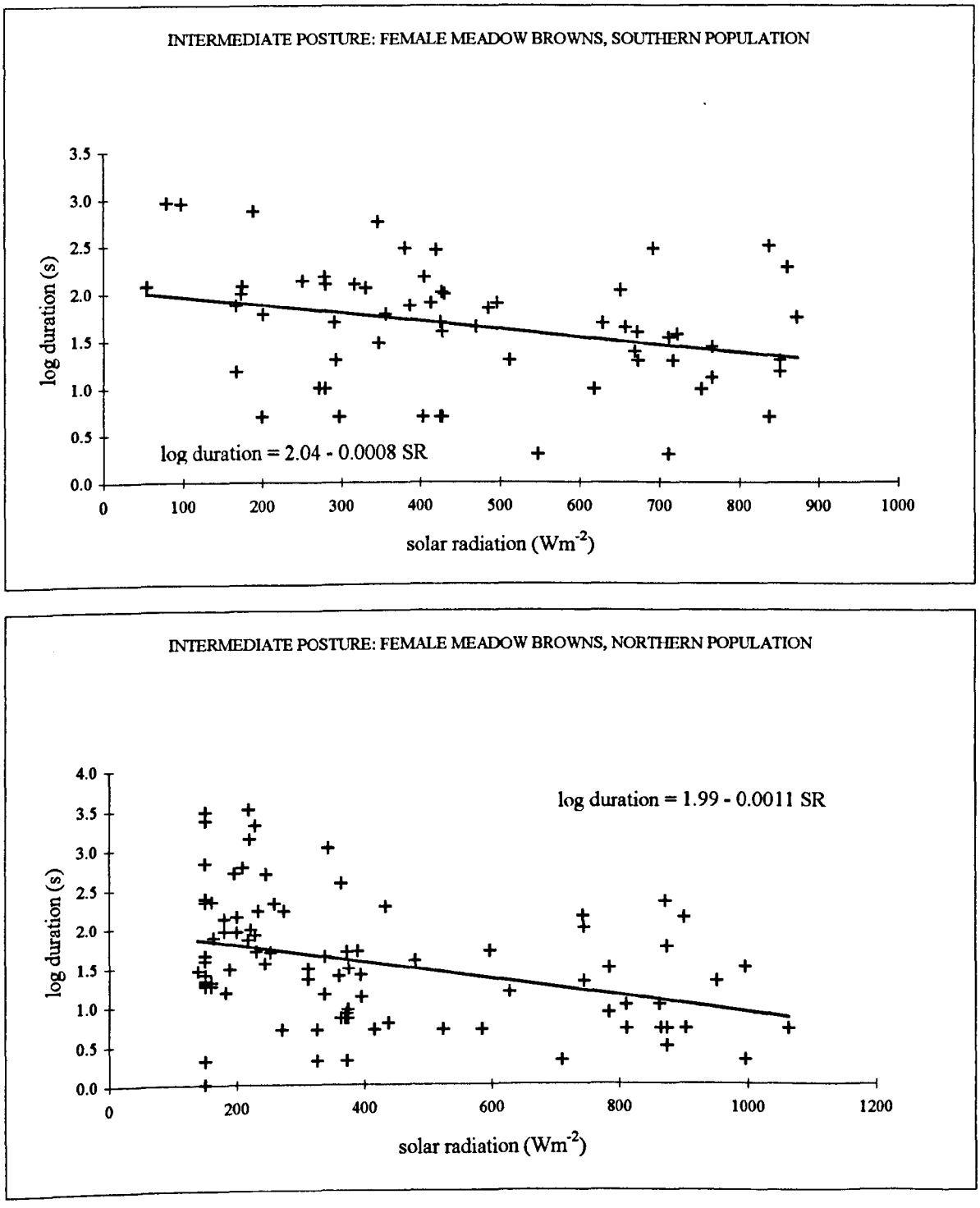


Figure 22. Duration of intermediate posture at different solar radiation intensities. Females *M. jurtina*, southern and northern populations.

9.3.6 Heights at which butterflies were alighted.

For each individual followed values for height at which butterflies were alighted (basking, heat avoiding or intermediate posture) were determined. These were then used to give population means for male and female butterflies in the southern and northern populations (Figure 23). Butterflies usually alighted at heights of within 1 m of the ground. Some would alight in bushes or trees at greater heights, but this was infrequent. The exception was in Bernwood Forest (south) when butterflies would alight in the trees to roost in the evening - these butterflies are excluded from the analysis. Figure 24 shows a frequency analysis of the distribution of heights at which butterflies were found basking, heat avoiding and intermediate posture in northern and southern populations. The height categories chosen correspond approximately to the vegetation structure:

0 - 2 cm	bare earth/rock, very short grass
2.1 - 5 cm	short grass and low herbaceous plants
5.1 - 40 cm	within longer grass and herbaceous layer
40.1 - 80 cm	top of longer grass and herbaceous layer
> 80 cm	shrubs and trees

Figure 24 shows that for the southern population, most males tended to alight at height of between 5 and 40 cm, with fewer alighting very low or very high. The majority of females in the south tended to alight at heights of between 0 and 2 cm, with progressively fewer found at greater heights. In the north, most males tended to alight at between 0 - 2 cm, with progressively fewer found at greater heights. Most females in the north were found basking at heights of 2 - 5 cm, were evenly distributed between 0 and 40 cm for intermediate posture and were usually found low down when heat avoiding. No butterflies were found alighted at heights of more than 30 cm in the north.

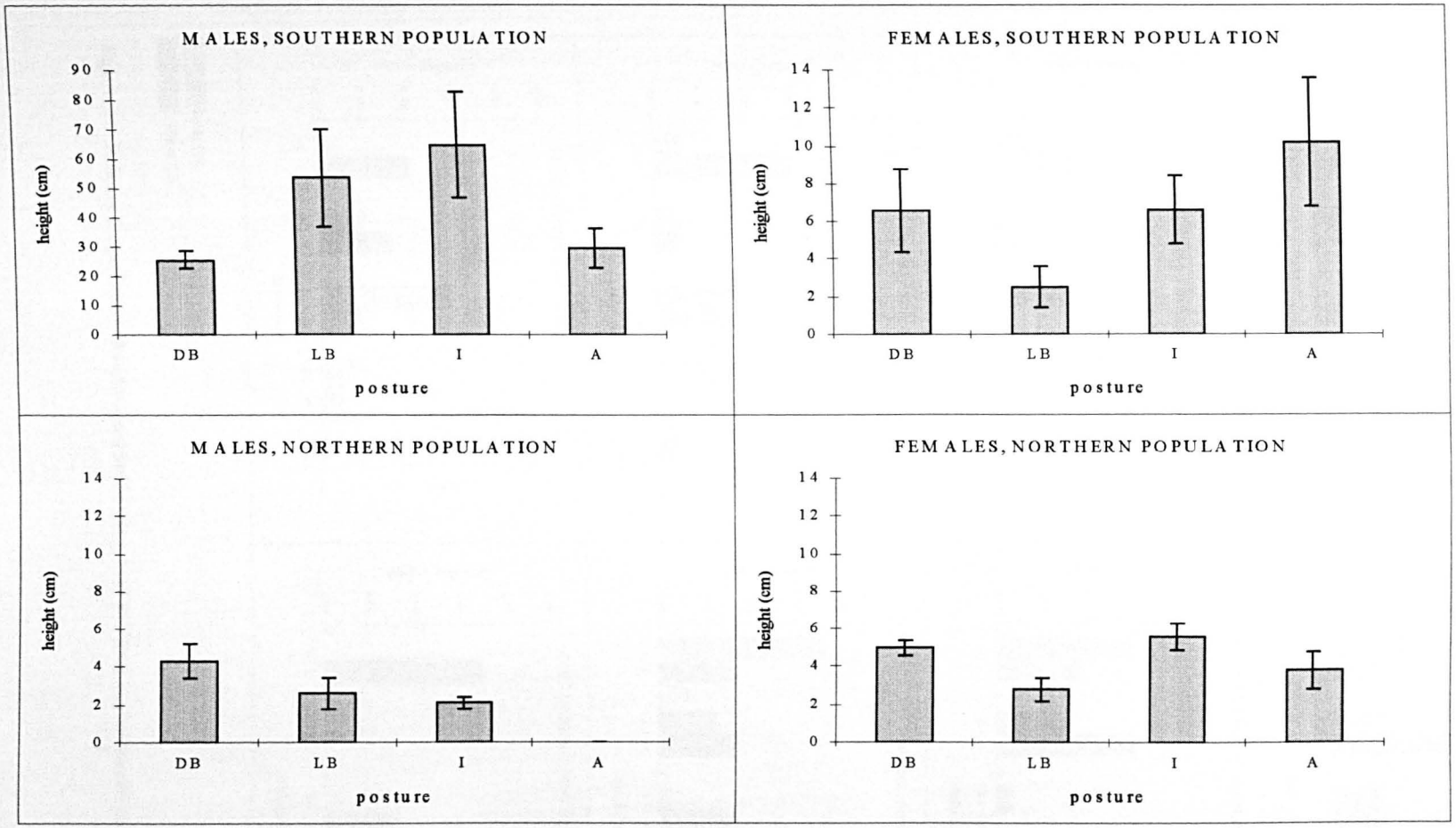


Figure 23. Mean height of basking, intermediate posture and heat avoiding.

DB = dorsal basking
LB = lateral basking
I = intermediate posture
A = heat avoiding

(NB note different y axis scale for males,south)

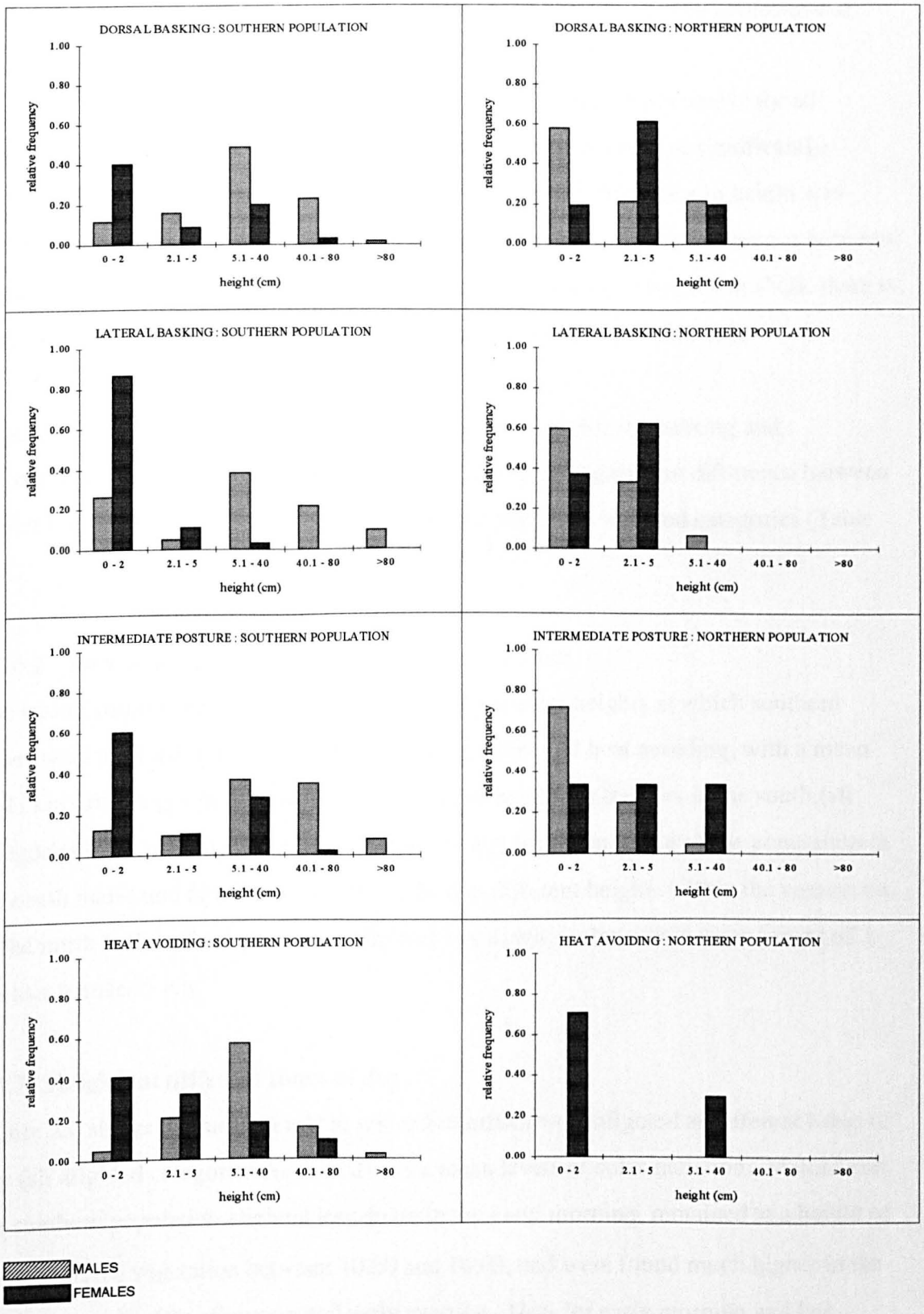


Figure 24. Relative frequencies of heights used for basking, heat avoiding and intermediate posture.

Differences between sexes and regions in mean height of basking, heat avoiding and intermediate posture.

Males in the south were found at significantly greater heights than females for all categories of alighted behaviour. Females in the north were found at significantly greater heights than males for intermediate posture, but the difference in height was small (2.1 cm for males, 5.6 cm for females). There is no significant difference between males and females in the north for any of the other categories of behaviour (N.B. there is only one datum for height of heat avoiding males in the north) (Table 18).

Males in the south were found at significantly greater heights for basking and intermediate posture than males in the north. There is no significant difference between females in the north and south for mean height of any of the alighted categories (Table 18).

9.3.6.1 Summary of heights at which butterflies alighted.

The main feature to emerge from these data is the greater heights at which southern males were alighted, for basking, intermediate posture and heat avoiding, with a mean of 41 cm (all categories of behaviour). The mean height for females in the south (all categories of behaviour) was 6 cm. This means that for the majority of the population in the south males and females would be alighted at different heights within the vegetation. In the north both males and females alighted low down; males with a mean height of 3 cm and females 5 cm.

9.3.7 Heights at different times of day.

Figure 25 shows the mean height at which butterflies were alighted at different times of day (all alighted categories combined), with mean levels of solar radiation. Males from the southern population alighted low down in the early morning, remained at a height of $\cong$ 20 cm in the vegetation between 10.00 and 14.00, and were found much higher in the vegetation in the late afternoon and early evening. Data for early morning and late afternoon are not available for southern females, which were found at a height of approximately 8 cm between 10.00 and 14.00 and then slightly lower ($\cong$ 4 cm) between 14.00 and 16.00.

Table 18. Height (cm) at which butterflies alighted (mean ± s.e.) with significance of differences: between southern and northern regions, and between sexes within each region. (two-tailed t-test, assuming unequal variance).

		MALES		*FEMALES*	
	ACTIVITY	*N*	*MEAN ± S.E.*	*N*	*MEAN ± S.E.*
SOUTHERN	*dorsal basking*	70	25.5 ± 3.0	25	6.6 ± 2.2
	lateral basking	43	53.5 ± 16.6	37	2.5 ± 1.2
	intermediate	55	64.7 ± 18.0	51	6.6 ± 1.8
	heat avoiding	44	29.6 ± 6.8	21	10.3 ± 3.4
NORTHERN	*dorsal basking*	38	4.3 ± 0.9	31	5.0 ± 0.4
	lateral basking	14	2.6 ± 0.8	8	2.8 ± 0.6
	intermediate	49	2.1 ± 0.3	48	5.6 ± 0.7
	heat avoiding	1	1.0	17	3.8 ± 1.0

	ACTIVITY			
COMPARISON	*dorsal basking (DB)*	*lateral basking (LB)*	*intermediate (I)*	*heat avoiding (A)*
southern ♂ - *southern* ♀	♂ > ♀ t = 5.14 ***	♂ > ♀ t = 3.07 **	♂ > ♀ t = 3.21 **	♂ > ♀ t = 2.54 **
northern ♂ - *northern* ♀	NS	NS	♀ > ♂ t = 4.40 ***	-
southern ♂ - *northern* ♂	south > north t = 6.86 ***	south > north t = 3.06 **	south > north t = 3.47 ***	-
southern ♀ - *northern* ♀	NS	NS	NS	NS

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant

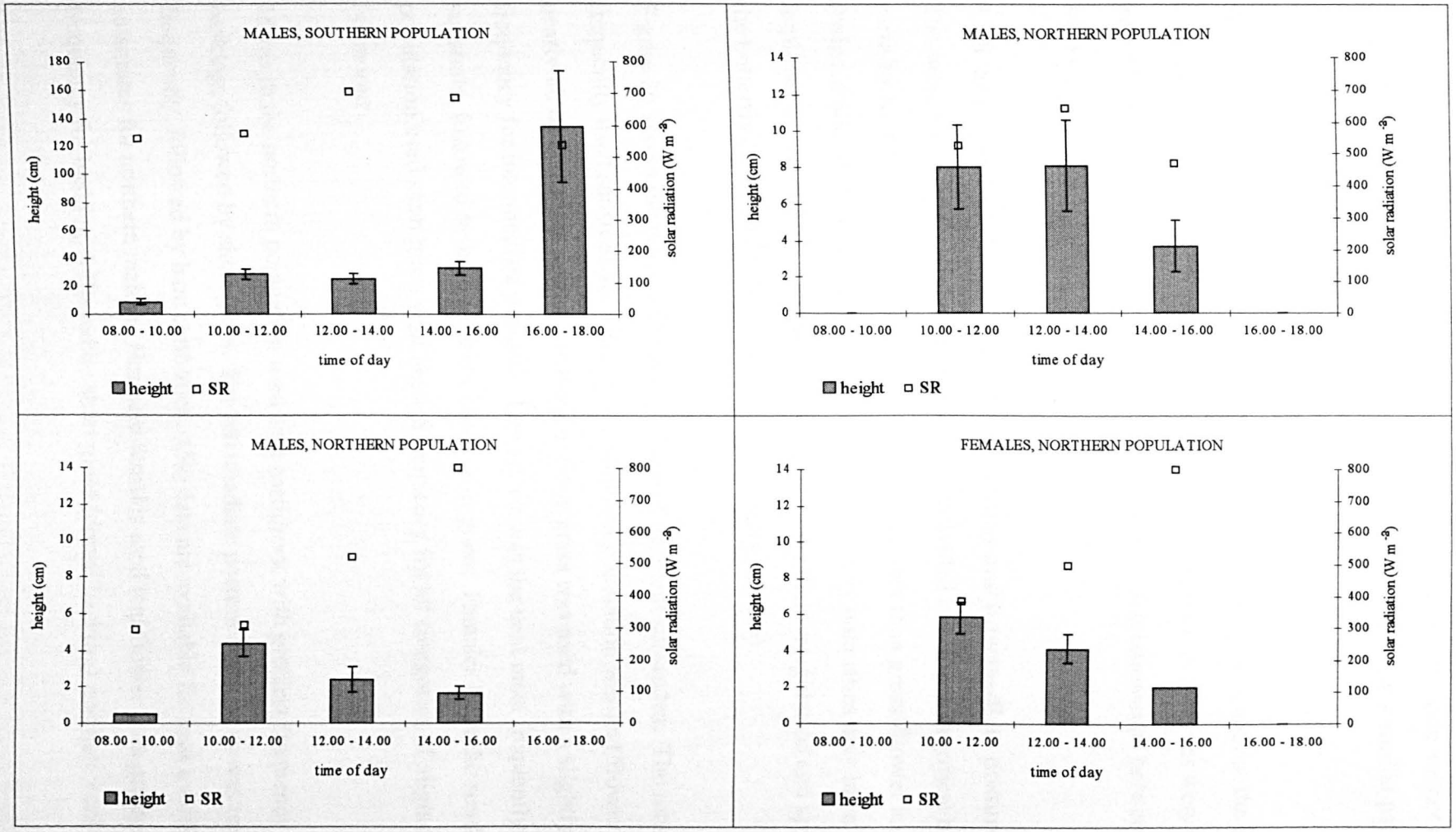


Figure 25. Mean height of alighted butterflies at different times of day and solar radiation intensity (SR).

(NB note different y axis scale for males,south)

Males from the northern population were also alighted very low in the vegetation early in the morning, with height increasing slightly later in the morning and then decreasing again during the afternoon. Data for early morning and late afternoon are not available for northern females, but for the times of day available they show a similar pattern to the northern males.

To see whether solar radiation intensity and T_a, which change throughout the day, influenced height at which butterflies were alighted, regression analyses were carried out of height against SR and T_a. There are no significant relationships between SR or T_a and height for either sex or region.

9.3.8 Substrates used for basking, heat avoiding and intermediate posture.

The substrates used by alighted butterflies were divided into five categories: bare earth/rock, short grass, long grass, leaf (dicot leaf, rather than grass)/flower and other (twigs, stalks, miscellaneous etc.). Substrates chosen by butterflies may have implications for both the associated microclimate for thermoregulation and apparency of the butterfly (which will also be influenced by wing posture).

Figure 26 shows the relative frequency of use of different substrates. The most frequently used substrate for males in the southern population was leaf/flower, for nearly all categories of alighted behaviour (long grass was used with a slightly higher frequency for intermediate posture). Long grass was the next most frequently used substrate, followed by bare earth/rock and short grass. Females from the southern population used short grass with highest frequency for all categories of alighted behaviour.

Males in the northern population used bare earth/rock with greatest frequency for basking, followed by short grass. For intermediate posture short grass was used most frequently, followed by bare earth/rock. (No data are available for heat avoiding substrates for northern males). Northern females used leaf/flower with greatest frequency for basking, followed by short grass (dorsal basking) and bare earth/rock

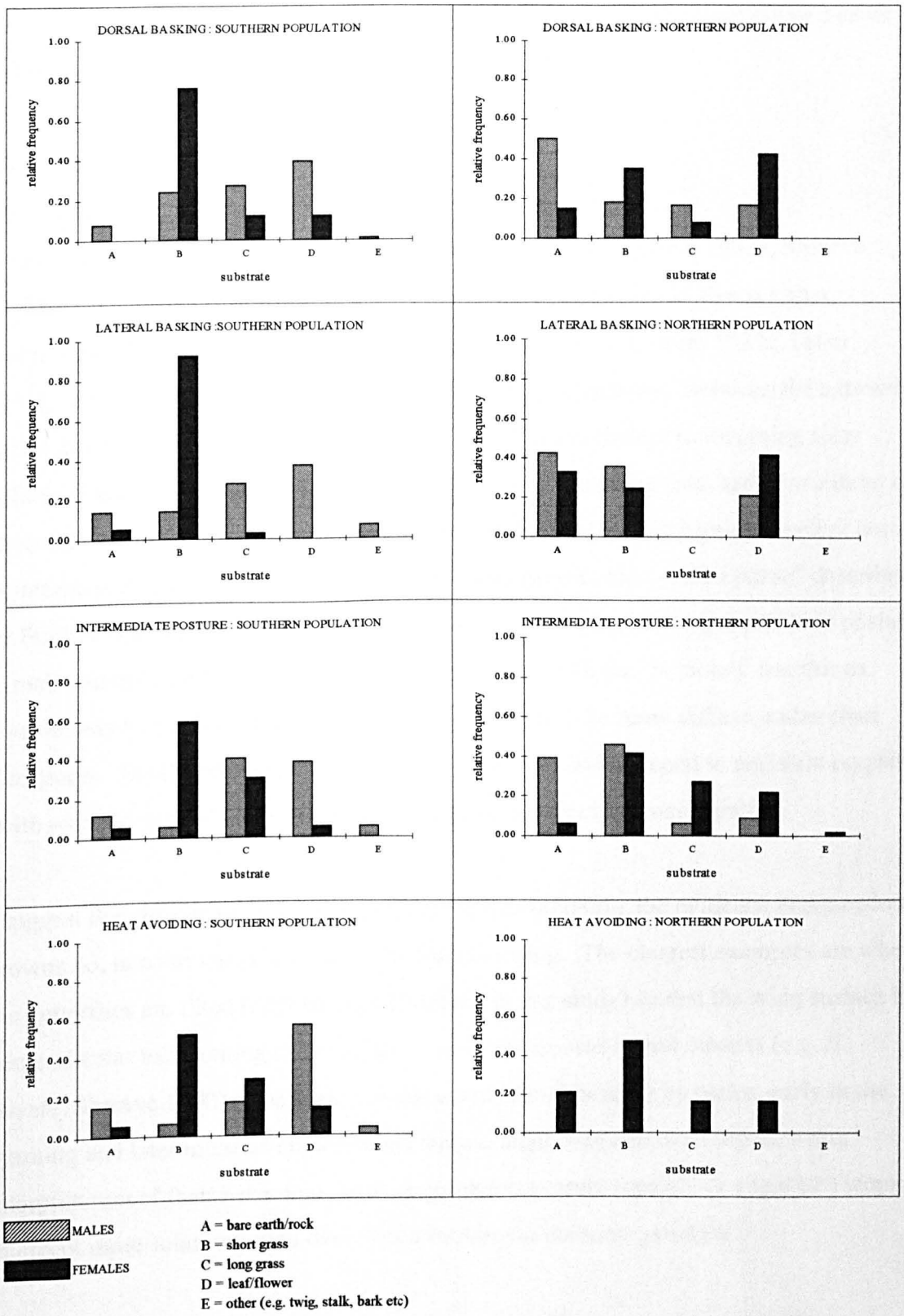


Figure 26. Relative frequencies of substrates used for basking, heat avoiding and intermediate posture.

(lateral basking). Short grass was used with highest frequency for intermediate posture and heat avoiding.

9.4 Discussion

Basking posture

Meadow browns are usually described as dorsal baskers (Brakefield 1982a, Shreeve 1992). When meadow browns are alighted with their wings closed, this is either described as resting, heat avoiding or roosting behaviour (Brakefield 1982a, Feber 1993). Here I interpret some of this behaviour as lateral basking. Between the extremes of full dorsal basking (wings fully open, orientated perpendicular to incoming solar radiation) and heat avoiding (wings closed, parallel to incoming solar radiation) there is obviously a wide range of postures which may be adopted, which I group together here as intermediate posture. Some of these may correspond to the "graded phase" described by Findlay *et al.* (1983) and Dreisig (1995), and representing fine adjustments to posture to maintain optimum body temperature in readiness for flight. In cloudy conditions, posture may be random, as incoming solar radiation will be more diffuse, rather than directional. In other cases the butterfly may be resting and the need to maintain crypsis (with only the ventral hindwing exposed) may be the primary consideration.

I suggest that despite the difficulties of correct interpretation, the evidence that meadow browns do, in some cases, use lateral basking is strong. The clearest examples are when the butterflies are tilted (22% of lateral basking in this study) so that the wing surface is perpendicular to incoming solar radiation, as in undisputed lateral baskers (e.g. *H. semele*, Shreeve 1990). The higher incidence of lateral basking by males, early in the morning and later in the afternoon when the sun angle was low, also supports this interpretation of their behaviour, as a wings-closed posture (upright or slight tilt) would intercept more solar radiation than dorsal basking in the same position.

Most butterfly species are described as adopting either dorsal *or* lateral basking postures (Clench 1966, Shreeve 1992, Shreeve & Dennis 1992). For butterflies to use both postures appears to be extremely uncommon. Heinrich (1986b) reports that *Agriades glandon* uses both dorsal and lateral basking, depending on the angle of the

sun. *A. glandon* showed a similar pattern to that described here for male *M. jurtina*, with the highest incidence of lateral basking occurring in the early morning and late afternoon. Heinrich suggests that this pattern of switching between lateral and dorsal basking at different times of day can be interpreted by referring to the butterflies' need to maintain a good field of vision for spotting potential mates. If lateral postures were adopted at a high sun angle, then the tilting required to maintain the wing surface perpendicular to incoming solar radiation, means that one eye would be pointing at the ground, so half the butterfly's field of vision would be obscured. The pattern of basking found in this study gives some support to this idea. When tilting was observed with lateral basking, this tended to occur when the sun angle was high (76% of tilting butterflies were observed between 11.30 and 14.00). The occurrence of decreased lateral basking around midday for male, but not for female butterflies also lends support to the idea that mate location requirements may be a contributory factor to the pattern found here.

The use of both basking postures by meadow browns may be another example of the behavioural flexibility of this species. This flexibility is also demonstrated by the additional use of the ground hugging posture, under conditions of low solar radiation, when warm air is trapped beneath the wings, making a significant contribution to body temperature excess (Kevan & Shorthouse 1970, Wasserthal 1975, Heinrich 1993).

Basking duration

The duration of basking in male butterflies (north and south) decreased with increasing solar radiation. Shreeve (1990) found that the length of time *H. semele* remained settled was related to substrate temperature (which was a function of substrate type and solar radiation intensity), and that *H. semele* remained settled for longest at lowest temperatures. Dreisig (1995) also found that the duration of basking in *H. semele* and *O. venata* decreased with increasing temperature. A similar pattern is found in *P. aegeria* (Shreeve 1984).

Duration of basking will depend on a number of factors. As SR intensity and T_a increase, the butterfly will warm to its optimum body temperature at a faster rate and so

will require less basking time. If butterflies are artificially constrained and remain in a basking posture at high SR intensity, their T_{th} will rapidly approach lethal temperatures (Heinrich 1993). Other factors affecting basking duration will include microhabitat temperatures which are influenced by the height and nature of the substrate (Shreeve 1984, Heinrich 1986b, Rutowski *et al.* 1994). Basking duration will also be influenced by the initial T_{th} of the butterfly, which will in turn be affected by the behaviour of the butterfly prior to basking.

Another factor influencing duration of basking (within the constraints of T_a and SR) might be the *function* of basking. In male butterflies the main function of basking is to raise thoracic temperatures to the required temperature for flight, or readiness for flight, with most flight activity being related to mate location (Heinrich 1986a, 1993, Ravenscroft 1994, Dreisig 1995, Van Dyck *et al.* 1997a, 1997b), but also for foraging and evading predators. Patrolling species, such as meadow browns, will usually alternate between basking (thoracic temperatures increase) and flight (thoracic temperatures decrease). Females spend a smaller proportion of their time in flight, but may still need to keep thoracic temperatures at a level at which flight is possible for much of the time (e.g. for escaping predators, foraging or locating egg laying sites). An additional function of maintaining a high thoracic temperature for females is that it increases the rate of egg maturation (Stern & Smith 1960). Shreeve (1984) found that the mean basking duration in *P. aegeria* was longer for females than for males. Here, northern *M. jurtina* females were found to bask for longer than northern males. Shreeve suggests that for *P. aegeria*, this may relate to egg production rates for females. The same argument could be applied here for female *M. jurtina*, especially for northern females which experience generally lower ambient air temperatures and fewer hours of sunshine.

Heights and substrates used for alighted behaviour

Several authors have demonstrated that perch height and substrate type are chosen primarily for favourable microclimates. Choice of height and substrate may change throughout the day and under different conditions of T_a and SR (Casey 1981, Shreeve 1984, 1990, Ravenscroft 1994). For male butterflies, perch height may also influence

male vision and hence chance of locating potential mates (Ravenscroft 1994). Here, northern males tended to use bare earth and rock (warm microclimate) for basking. All butterflies tended to be found higher in the vegetation at around midday. This is probably due to the increased temperature at ground level which was generally several degrees higher than that at vegetation height (Chapter 4, section 4.2). In the hottest part of the day, the microclimate nearer the ground may have been too warm for prolonged perching.

The generally greater heights at which southern males were found, compared to southern females and northern butterflies of both sexes, may relate to their use of perches as part of their mate location strategy, with the greater height increasing the distance over which potential mates could be seen. Ravenscroft (1984) found that *C. palaemon* used perches, to locate mates, at a height of approximately 40 - 50 cm most of the time, a similar height to that found here for southern males. Brakefield (1982a) found that male *M. jurtina* tended to congregate on an elevated bank and suggests that this was to increase the distance at which females could be detected.

Overall, the difference in alighted behaviour between northern and southern regions is perhaps less pronounced than for other behavioural categories (such as active flight of males). These results again demonstrate how behaviour is constrained by weather conditions (e.g. butterflies need to bask for longer at low SR intensity). They also provide further evidence of the flexibility of behaviour in *M. jurtina*, especially the observation that they can adopt both dorsal and lateral basking postures.

CHAPTER 10. FEEDING BEHAVIOUR

10.1 Introduction

Although adult butterflies emerge from the pupa with a reserve of energy built up from larval feeding, stored in the fat-body of the abdomen (Ford 1945), most still require food in the adult stage to achieve maximal reproductive output and longevity. Adult meadow browns feed on floral nectar. Nectar consists primarily of sugars (mono- di- and oligosacharides) dissolved in water, but may also contain amino acids, proteins, enzymes and alkaloids (Scoble 1992). The dilution and abundance of floral nectar varies throughout the day, being most abundant and dilute in the early morning and more concentrated and scarce in the afternoon. The concentration and quantity of nectar will also vary with weather conditions and activity of other nectar feeders (Shreeve, 1992, Scoble 1992). Watt *et al.* (1974) and Kingsolver & Daniel (1979), suggest that the optimum sugar concentration in nectar for most butterflies is 20 - 25% (which accords with the concentrations found for feeding by *Colias*). Pivnick & McNeil (1985), however, found that the European skipper (*Thymelicus lineola*) consistently feeds on nectar with sugar concentrations of 40 - 65% in the field. In the laboratory they found that a concentration of 40% gave the optimum uptake rate. Boggs (1988) found peak rates of ingestion occurred at sucrose concentrations of between 30% and 40%, with rates of ingestion varying with sex, age and size in *Speyeria mormonia.*

Although adult energy requirements are not high (Pivnick & McNeil 1985, Scoble 1992), adult feeding may still be important for obtaining energy and water (which may lead to individuals selecting more dilute nectar sources under conditions of water stress) as well as using carbohydrates and amino acids for sperm and egg production. The last may be particularly important for female meadow browns, whose eggs mature after emergence. In *T. lineola*, for example, feeding on sucrose by females increased fecundity by 27 times (Pivnick & McNeil 1985). Feeding in adult females increased longevity and egg production in *Colias p. eurytheme* (Stern & Smith 1960) and in *Euphydryas editha* (Murphy *et al.* 1983). In many species males also acquire nutrients as adults help in the production of the "nuptial gift", a mixture of nutrients passed to the female along with the sperm during mating (Oberhauser 1997, Karlsson *et al.* 1997).

The different energetic demands of males and females and the different requirements for sperm and egg production means that the nutritional requirements of the sexes may differ.

Meadow browns feed on a wide variety of nectar sources (Pollard 1981, Brakefield 1982a, Shreeve 1989, Feber 1993). Energetic costs, nutritional rewards, handling time and flower density may influence whether butterflies show flower constancy, with individuals showing a preference for particular species of flowers, as has been demonstrated for other species of butterfly (Goulson & Cory 1993, Goulson, Ollerton & Sluman 1997, Goulson *et al.* 1997, Kandori & Ohsaki 1996, Weiss 1997).

Overall, foraging patterns are expected to be influenced by a range of factors which may include; age and sex of the individual butterflies, time of day and weather conditions (which will influence the concentration and quantity of nectar), learned preferences and abundance of nectar plants. Willmer (1983) has also show that in other ectothermic insect species (flies and bees) timing and duration of foraging is strongly influenced by solar radiation intensity, which effects thermal constraints and opportunities as well as nectar rewards.

A number of questions are asked in relation to feeding in meadow browns:

1. What are the conditions of temperature and solar radiation under which feeding takes place, and do these differ between the sexes and between the southern and northern populations?
2. Does the duration of feeding bouts differ between the sexes and between the regions?
3. Does the duration of feeding bouts and percent of time allocated to feeding vary throughout the day?
4. Is the duration of feeding bouts influenced by temperature and solar radiation?
5. What are the wing angles adopted during feeding (e.g. are butterflies basking opportunistically during feeding)?
6. Do meadow browns exhibit flower constancy?

10.2 Methods

Data from following individual butterflies in both southern and northern sites are used for analyses of feeding behaviour. For each feeding bout the time, duration, wing angle and species of flower used were recorded (see Chapter 3, section 3.2). Each bout of feeding was taken as the time spent feeding on a single plant, but including moving between inflorescences on the same plant. Distance flown between flowers was estimated for individuals in the southern population but not for the northern population (the rough ground made this too difficult).

10.3 Results

Using data from all individuals followed, feeding behaviour accounted for 24.1% of total contact time for male meadow browns from the southern population (69 individuals (N_i) 701 contact minutes (N_c)). Feeding accounted for 21.7% of total contact time for southern females (N_i = 27, N_c = 443), 11.6% for northern males (N_i = 35, N_c = 489), and 8.2% for northern females (N_i = 22, N_c = 678). These values represent the percent of time allocated to feeding at the population level. The difference between southern and northern males was significant, but there was no significant difference in the percent of time allocated to feeding by southern and northern females, or by males and females within each region (data arcsine transformed to approximate normality), Table 19.

Table 19. Mean percent of contact time allocated to feeding, with comparisons between the sexes and region (two-tailed t. tests, assuming unequal variance, data arcsine transformed). *Maniola jurtina*, southern and northern populations.

	mean % time feeding	*n (number of individuals)*	
♂ SOUTH	24.1 ± 3.9	69	
♀ SOUTH	21.7 ± 6.7	27	
♂ NORTH	11.6 ± 3.3	35	
♀ NORTH	8.2 ± 3.9	22	
comparison	t	df	P
♂ SOUTH - ♀ SOUTH	0.25	94	0.80
♂ NORTH - ♀ NORTH	0.63	55	0.53
♂ SOUTH - ♂ NORTH	2.65	102	0.009
♀ SOUTH - ♀ NORTH	1.84	47	0.07

Butterflies used a variety of flower species for feeding. In the southern population the most frequently visited species were field scabius (*Knautia arvensis*), spear thistle (*Cirsium vulgare*), creeping buttercup (*Ranunculus repens*), common spotted orchid (*Dactylorhiza fuchsii*), and dwarf thistle (*C. acaule*). Other species visited included; knapweed (*Centauria* spp.), blackberry (*Rubus fruticosus*), rough hawkbit (*Leontodon hispidus*), smooth hawk's beard (*Crepis capillaris*), marjoram (*Origanum vulgare*), bird's foot trefoil (*Lotus corniculatus*), self-heal (*Prunella vulgaris*), centaury (*Centaurium erythraea*), white clover (*Trifolium repens*), ox-eye daisy (*Chrysanthemum leucanthemum*) and meadow vetchling (*Lathyrus pratensis*). In the northern population the most frequently visited species were thyme (*Thymus praecox*), thistles (*Cirsium* spp.) and self-heal. Other species visited included; white and red clover, creeping buttercup, eye bright (*Euphrasia officinalis*) knapweed and harebell (*Campanula rotundifolia*).

10.3.1 Temperature and Solar Radiation at which feeding was initiated

Male *M. jurtina* from the southern region initiated feeding at a mean T_a of 22.5 ± 0.3°C, which is significantly higher than the T_a at which southern females initiated feeding (20.0 ± 0.6°C) ($t = 3.75$, $df = 57$, $P < 0.001$). Males from the northern population also initiated feeding at higher T_a than females (20.8 ± 0.3°C and 19.2 ± 0.4°C respectively) ($t = 3.43$, $df = 27$, $P = 0.001$). Males from the southern population initiated feeding at significantly higher T_a ($t = 3.96$, $df = 61$, $P < 0.001$) and SR ($t = 3.46$, $df = 61$, $P < 0.001$) than males from the northern population, but there is no significant difference in T_a ($t = 1.05$, $df = 21$, $P = 0.29$) or SR ($t = 1.73$, $df = 21$, $P = 0.08$) between females from the southern and northern populations.

Since active flight is usually required to locate nectar sources, the T_a and SR levels at which feeding took place are compared with those for active flight. There is no significant difference between the T_a and SR levels for feeding and active flight. This suggests that although feeding itself is unlikely to be as energetically expensive as

active flight, conditions of ambient air temperature and solar radiation must usually be compatible with the requirements for active flight so that the butterflies can locate suitable sources of nectar.

10.3.2 Duration of feeding bouts

The mean duration of feeding bouts varied from 76.3 ± 17.5 s (northern males) to 133.8 ± 49.5 s (southern females). There is no significant difference in mean duration of feeding bouts between males and females or between southern and northern populations.

10.3.2.1 Percent of time allocated to feeding and duration of feeding bouts at different times of day

Time of day was divided into four periods: 08.00 - 12.00, 12.00 - 14.00, 14.00 - 16.00, >16.00. There is insufficient data for the northern population for each period, so only southern butterflies are used in this analysis.

The percent of time allocated to feeding by male *M. jurtina* remained at approximately 30% of total contact time during the morning and earlier part of the afternoon. The percent of time allocated to feeding increased in the late afternoon/early evening to approximately 80% of total contact time. Females also allocated approximately 30% of their time to feeding in the morning, this then fell to 9% at midday and rose throughout the afternoon to approximately 90% in the late afternoon/early evening (Table 20, Figure 27a).

Duration of feeding bouts was shortest at around midday, for both males and females, increasing to maximum in the afternoon for females (although this data point should be viewed with caution as it is based on only 3 feeding bouts) and late afternoon/early evening for males. Mean solar radiation levels fell sharply in the late afternoon/early evening, whereas mean ambient temperatures gradually increased throughout the day, to a maximum in the late afternoon/early evening (Table 20, Figure 27b).

Table 20. Mean percent of time allocated to feeding and mean duration of feeding bouts at different times of day. *M. jurtina* , southern population.

	MALES				FEMALES			
Time	*total contact mins*	*mean % time feeding*	*mean duration feeding (s)*	*n (feeding bouts)*	*total contact mins*	*mean % time feeding*	*mean duration feeding (s)*	*n (feeding bouts)*
08.00 - 12.00	88.1	29.6 ± 5.4	107.7 ± 30.4	26	75.2	28.7 ± 3.3	58.3 ± 8.8	20
12.00 - 14.00	248.0	29.3 ± 4.5	44.1 ± 8.7	78	92.8	9.1 ± 3.6	44.1 ± 7.6	10
14.00 - 16.00	96.2	34.3 ± 7.3	133.9 ± 20.8	15	47.3	59.7 ± 6.1	565.0 ± 34.9	3
>16.00	81.2	77.7 ± 4.0	228.1 ± 25.0	14	25.6	91.4 ± 1.2	235.0 ± 19.9	6

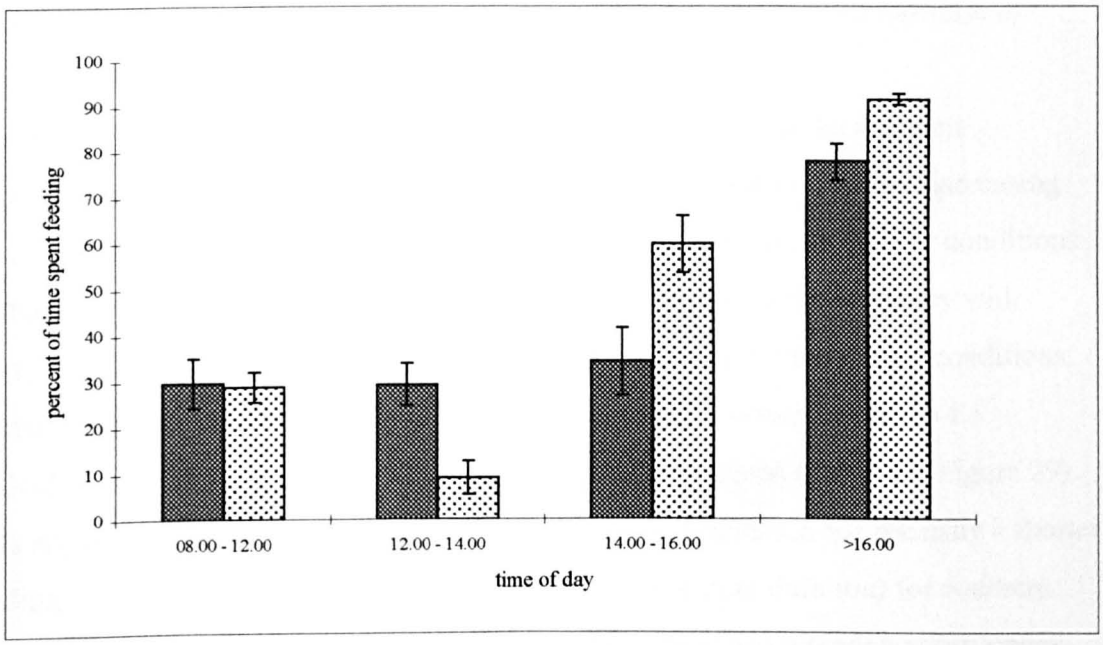


Figure 27a. Mean percent of time spent feeding

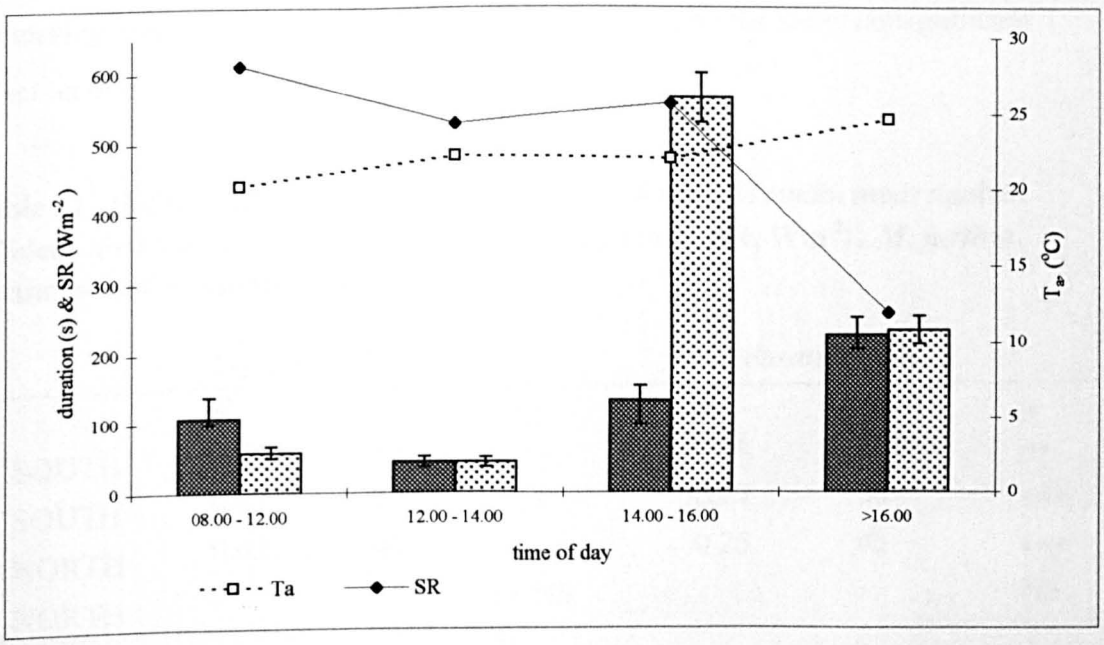


Figure 27b. Mean duration of feeding bouts, with mean solar radiation (SR) and ambient air temperature (T_a)

male

female

Figure 27. Mean percent of time spent feeding and mean duration of feeding bouts at different times of day. *Maniola jurtina*, southern population.

10.3.2.2 Influence of ambient air temperature and solar radiation on duration of feeding bouts

Males and females from the southern population and males from the northern population show a tendency for duration of feeding bouts to decrease with increasing levels of solar radiation - i.e. individual feeding bouts are shorter in sunnier conditions (Table 21, Figure 28). Males from both regions also show a similar tendency with increasing levels of T_a - i.e. individual feeding bouts are shorter in warmer conditions. Females from the southern population show the opposite tendency with T_a - i.e. individual feeding bouts tend to be longer in warmer conditions (Table 21, Figure 29). This apparent discrepancy between the influence of SR (increased SR intensity - shorter feeding bout duration) and T_a (higher T_a - longer feeding bout duration) for southern females may be due to the influence of time of day. There was a tendency for longer feeding bouts in the late afternoon and early evening, when solar radiation levels were decreasing, but ambient temperature was still high. T_a and SR show no significant effect on duration of feeding bouts for northern females.

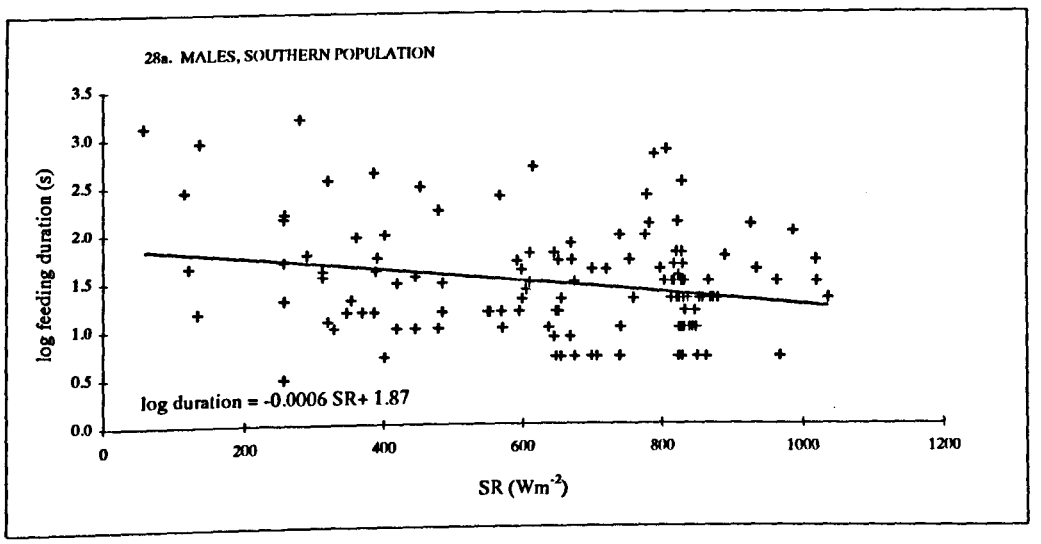
Table 21. Regression of duration of feeding bouts (log_{10} transformed) against ambient air temperature (T_a, °C) and solar radiation (SR, Wm^{-2}). *M.jurtina*, northern and southern populations.

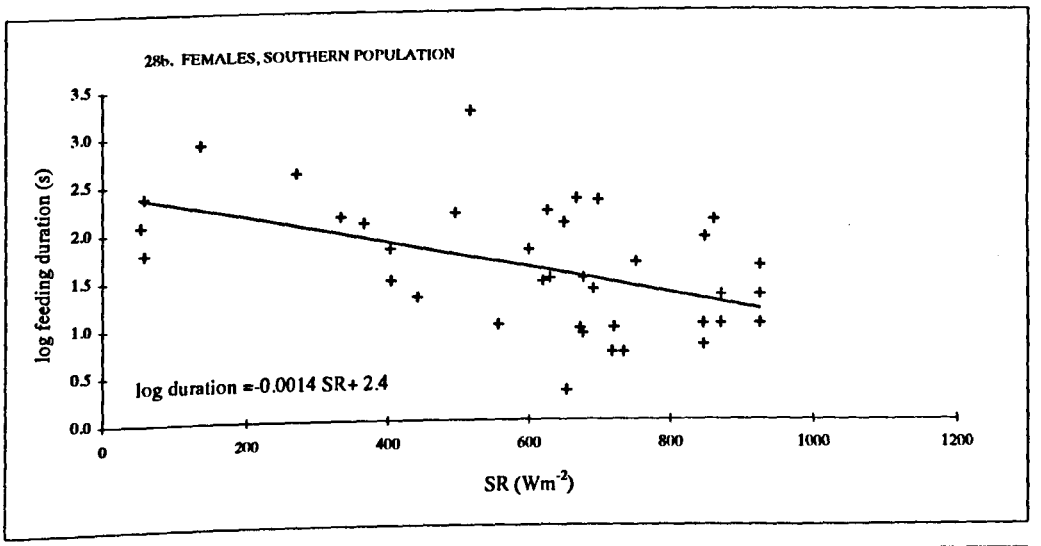
	log duration / T_a			*log duration / SR*		
	r^2	df	p	r^2	df	p
♂ SOUTH	0.04	131	*	0.06	131	**
♀ SOUTH	0.09	38	*	0.27	38	***
♂ NORTH	0.02	45	**	0.25	45	***
♀ NORTH	-	-	NS	-	-	NS

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant

10.3.3 Feeding and wing angles

During feeding butterflies would typically move around on the flower head while probing with their proboscis to extract the nectar. Three wing angles were observed for feeding butterflies: wings closed (0°), wings variable (opening and closing) and wings open (>160°).





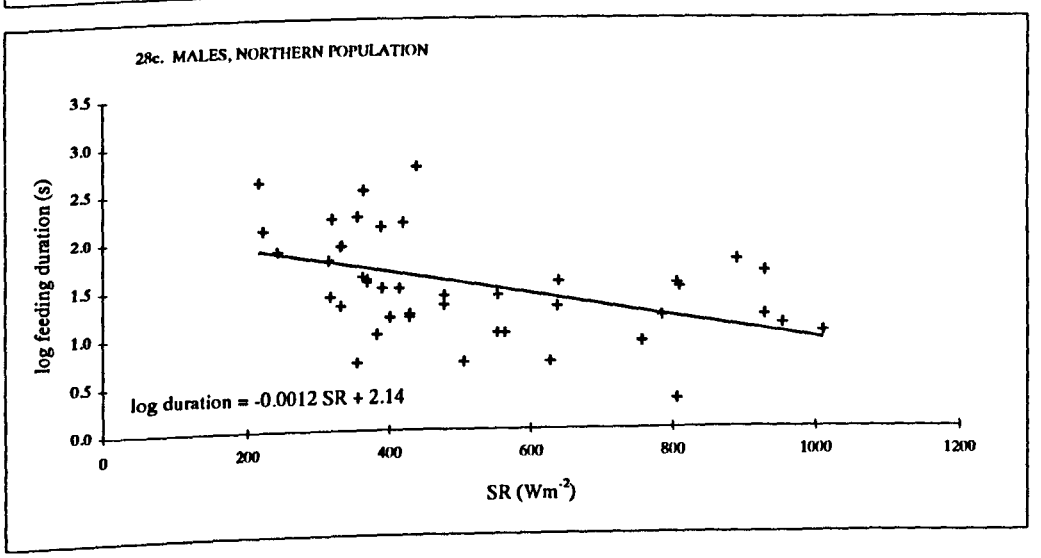
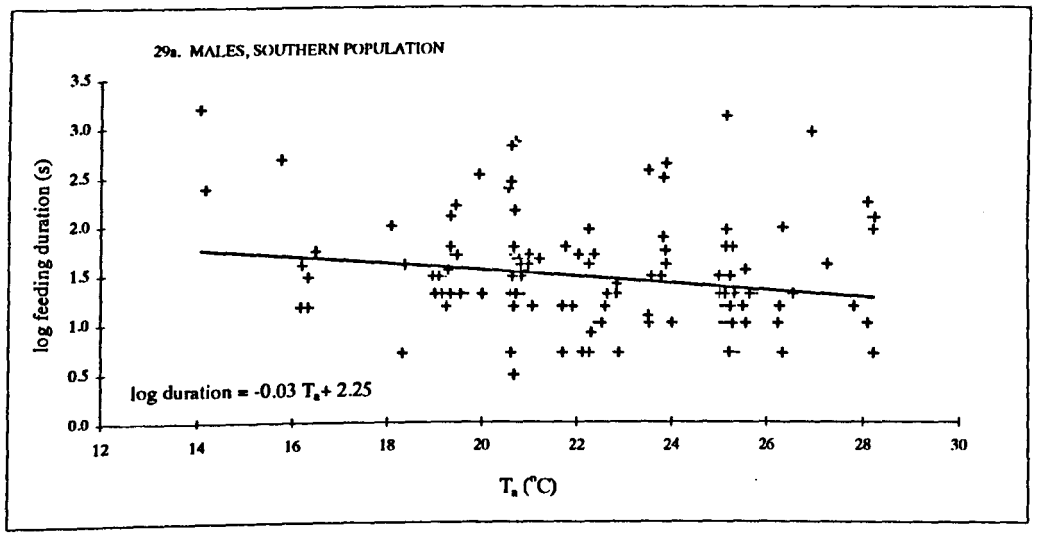
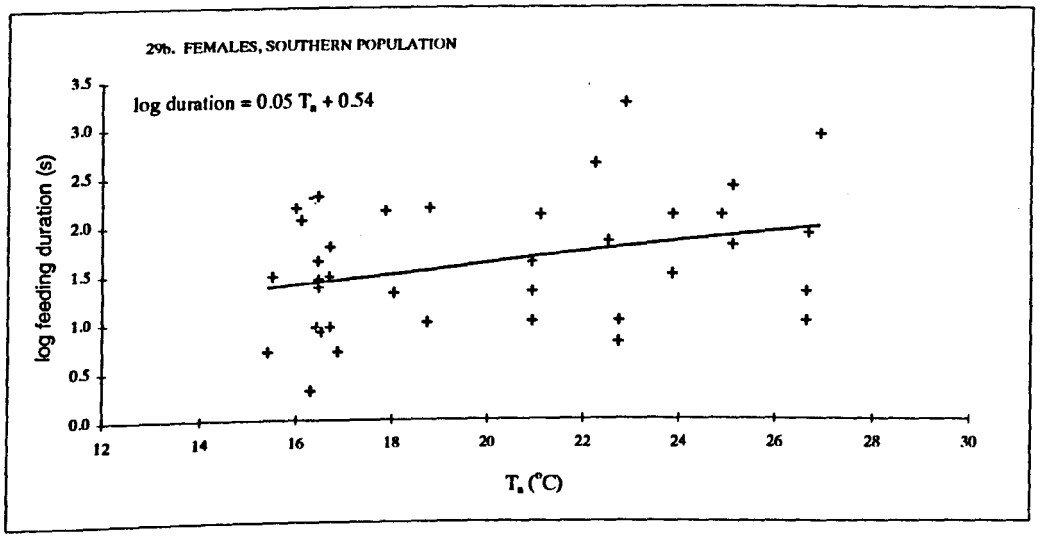


Figure 28. Duration of feeding bouts in relation to solar radiation (SR); male and female *Maniola jurtina*, southern population and male *M. jurtina*, northern population.





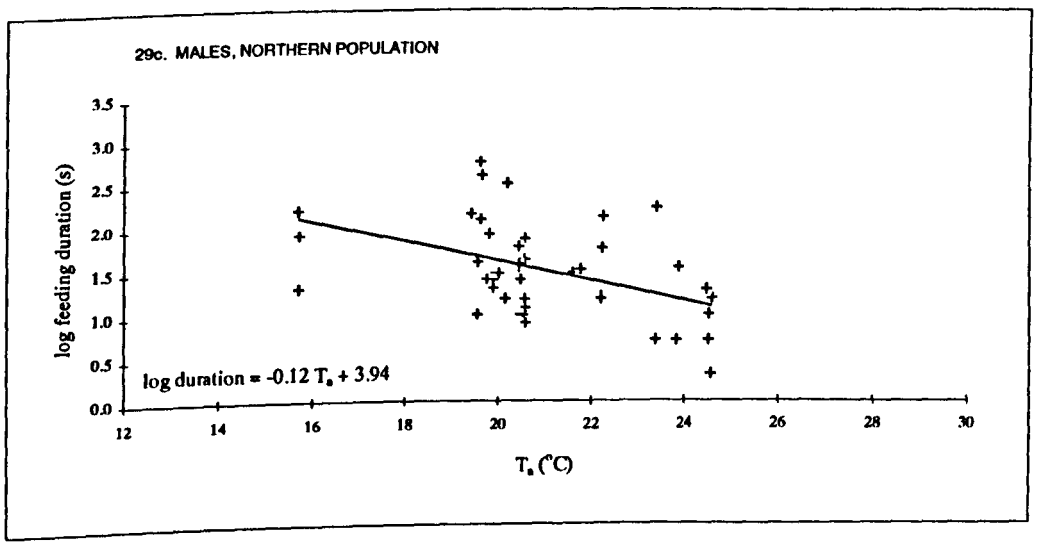


Figure 29. Duration of feeding bouts in relation to ambient air temperature (T_a); male and female *Maniola jurtina*, southern population and male *M. jurtina*, northern population.

The orientation of the butterflies in relation to solar radiation was not constant, but changed as the butterfly moved around the flower head. If butterflies were basking opportunistically during feeding, it is expected that they would adopt an open wing posture, as this would be more likely to absorb solar radiation while the butterfly moved around than would a closed wing posture (where the wings would be parallel to incoming solar radiation for a proportion of the time). If defence was of greater importance during feeding (primary defence - cryptic ventral hind wing exposed, eye spot covered; secondary defence - cryptic ventral hind wing exposed, eye spot visible) then the butterfly is expected to adopt a closed wing posture. Variable wing posture (wings opening and closing) might indicate "fine-tuning" during thermoregulation, or possibly some form of signalling.

Butterflies feeding with their wings closed always had the forewing eye spot either exposed or half covered. In nearly all butterflies the wings closed posture was adopted with the highest frequency. This posture was adopted in 89% of feeding bouts for southern males, 78% for southern females, 66% for northern males and 50% for northern females. Variable wing posture was adopted with the next highest frequency, followed by wings open (apart from northern males, where variable and wings open postures were both adopted with equal frequency, 17% of feeding bouts). Northern males and females adopted the wings open posture with higher frequency than southern males and females. Northern females adopted the variable wing posture 35% of the time - higher frequency than either northern males or southern males and females (Figure 30).

10.3.4 Flower constancy

Flower constancy (a preference for feeding on species which have previously been visited) may lead to decreased "handling time" when locating and extracting nectar. Data on flower species density were not recorded in this study, so a full analysis of factors related to flower constancy is not possible. However, data on sequences of visits to different flower species, duration of feeding and distance travelled between flowers (for the southern population) were recorded, allowing some analysis of flower constancy to be undertaken.

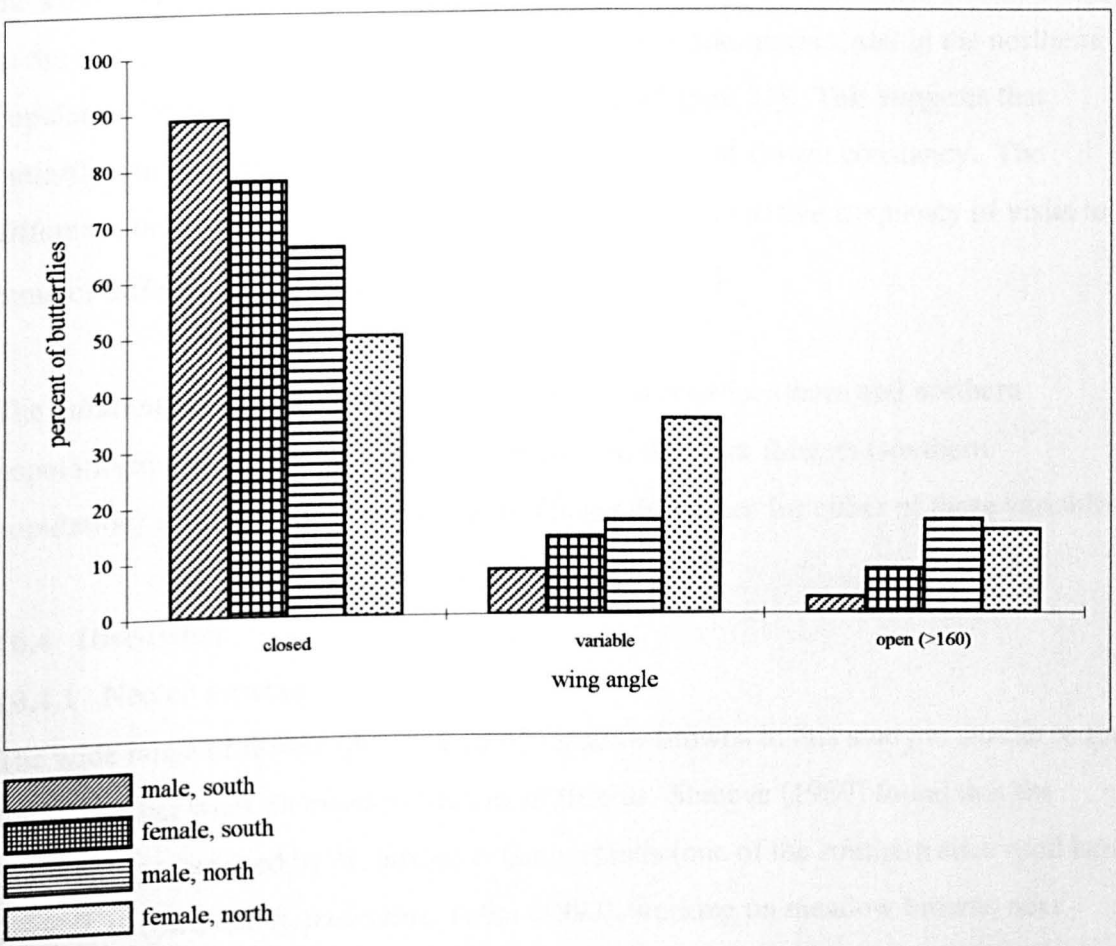


Figure 30. Wing angles of feeding butterflies: *Maniola jurtina* , southern and northern populations: Percent of butterflies feeding with wings closed, variable (opening & closing) or open (>160°).

If handling time is an important variable influencing flower constancy, then it is suggested that butterflies living in marginal conditions (where time available for essential activities may be limited) would be expected to exhibit a greater degree of flower constancy than those living in more favourable conditions (although other factors such as energetic costs, flower density and nutritional rewards may also influence flower constancy – see above).

Visits to flowers were classified as "same" (same species as previous flower visited) or "different" (different species to previous flower visited). Butterflies from southern and northern population (males and females combined) were more likely to visit a flower of the same species to that visited on the previous occasion than to visit a different species. In the southern population 64% of visits were to the same species, and in the northern population 75% of visits were to the same species (Figure 31). This suggests that butterflies in both populations were exhibiting a degree of flower constancy. The difference between southern and northern populations in relative frequency of visits to same or different flowers is not significant ($\chi^2, P > 0.05$).

The duration of feeding on the same or different flowers (southern and northern populations) and the distance travelled to same or different flowers (southern population) is compared. There is no significant difference for either of these variables.

10.4 Discussion

10.4.1 Nectar sources

The wide range of flower species used by meadow browns in this study is similar to that found in other work on meadow browns in Britain. Shreeve (1989) found that the preferred species used by *M. jurtina* at Grangelands (one of the southern sites used here) were *O. vulgare* and *R. fruticosus*. Feber (1993), working on meadow browns near Oxford, found the preferred flowers were *K. arvensis, Centauria* spp., *Cirsium* spp. and *Carduus* spp. Pollard (1981) records meadow browns as also feeding on *R. repens, T. repens, P. vulgaris* and *Ligustrum vulgare*. Brakefield (1982a) reports that *Cirsium* spp. were the most frequently used nectar sources. The range of species used varies throughout the flight period, depending on location and availability.

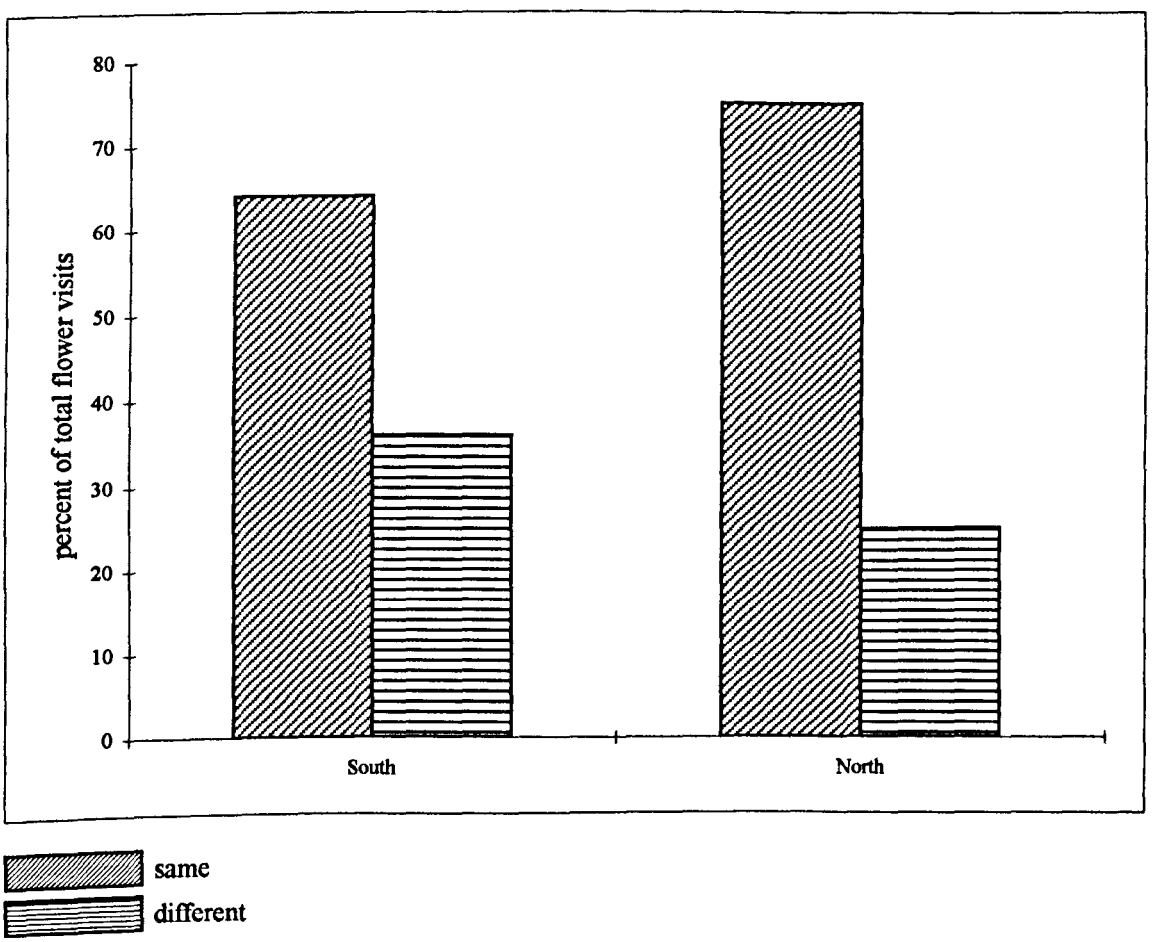


Figure 31. Percent of flowers visited which were the same as, or different from previous flower species visited

10.4.2 Percent of time allocated to feeding and duration of feeding bouts

Males and females

The percent of time allocated to feeding by southern *M. jurtina* (24% for males and 21% for females) was less than that found by Feber (1993) who reports 34% for males and 44% for females, in a study of meadow browns near Oxford. In this study feeding bouts of southern females were of longer duration than those of males (although the difference is not statistically significant). Brakefield (1982a) found that females fed more frequently and for longer duration than males. Pivnick & McNeil (1985) also found that female *T. lineola* fed for longer than males. Here, the northern population did not fit this general pattern, with females allocating less time to feeding, with feeding bouts of similar duration to that of males. Wiklund & Ahrberg (1978) found no significant difference in the feeding duration of male and female *A. cardamines*. They found that male and female *A. cardamines* flew for equal amounts of time, so attributed their similar feeding patterns to similar energy requirements. This argument does not seem to apply here, as if time spent in flight correlated with energy requirements and hence time allocated to feeding, then we would expect male meadow browns to have higher energy requirements and so spend more time feeding.

Here, the demands of mate location appear to take priority for males (males from both southern and northern populations allocated a higher percent of time to flying than feeding), with feeding occurring opportunistically between patrolling flights and within the constraints of thermoregulatory requirements (such as basking). For females, who spend a higher proportion of the time alighted, rather than flying, feeding is more likely to occur as a "priority" purpose of flight, rather than opportunistically in the course of other flight related activity.

Time of day

In the southern population there was a tendency for the percent of time allocated to feeding and the duration of feeding bouts to increase in the late afternoon and early evening. The longest duration feeding bouts were observed in Bernwood Forest on a bramble bush, in the early evening, prior to butterflies flying into the trees to roost. Here meadow browns and a number of other species (*A. hyperantus, P. tithonus* and

Ladoga camilla) were all seen feeding for prolonged periods on the same bush. Under these conditions, solar radiation intensity was probably too low for effective basking to allow active flight, but ambient air temperatures were still high enough for moderate activity. Under these conditions, prolonged feeding may have been the most effective use of time.

Feeding duration, T_a and SR

The general tendency was for duration of feeding bouts to decrease with higher T_a and SR. Pivnick & McNeil (1985) demonstrated that for *T. lineola*, under laboratory conditions, rate of uptake of sucrose solution was faster at 35°C than at 25°C. They found that the faster uptake at higher temperatures was due to the reduced viscosity of the sucrose solution and to the increased power output of the cibarial pump (the structure associated with pumping liquid up through the proboscis), mediated by the pressure changes induced by the pump. From this they developed a general model which is applicable to all nectar feeding insects with sucking mouth parts. If this model applies to meadow browns then the combination of high SR and T_a, leading to decreased viscosity of nectar and increased rate of uptake, would help account for the shorter feeding duration at higher T_a and SR, with the butterflies being able to take in a given volume of nectar in a shorter period of time.

Another factor here is the potentially conflicting requirements of different activities (e.g. whether to feed or fly) to which the butterflies must allocate their time. Conditions of high T_a and SR are favourable for flight, so butterflies take advantage of this to engage in activities other than feeding, such as mate location.

10.4.3 Flower constancy

Although a wide variety of different flower species were used by meadow browns, individual butterflies in both southern and northern regions showed a tendency towards flower constancy - being more likely to visit a flower of the same species as visited previously, than to visit a flower of a different species.

Weiss (1997) demonstrated an innate colour preference (for yellow flowers) in the pipevine swallowtail (*Battus philenor*), but they were also able to learn and adjust foraging efforts in response to floral rewards. Kandori & Ohsaki (1996) found that in *Pieris rapae*, some individuals had a strong preference for one species of flower, whereas other individuals would use several. They found that handling time became shorter as butterflies learnt to extract nectar more efficiently. Goulson, Ollerton & Sluman (1997) and Goulson *et al.* (1997) also suggest that decreased handling time contributes to flower constancy in *Thymelicus flavus* and *P. rapae* (Goulson & Cory 1993). They suggest that switching to a different species is more likely to occur when the preferred species is scarce or the rewards are low.

10.4.4 Foraging strategies

Schoener (1971) distinguishes *time minimizers* ("fitness is maximised when time spent feeding to gather a given energy requirement is minimized") from *energy maximizers* ("fitness is maximised when net energy is maximised for a given time spent feeding"). Data presented here supports the view that meadow browns are behaving as time minimisers rather than energy maximisers. Data to support this includes the decreased duration of feeding bouts under warm and sunny conditions and the occurrence of flower constancy*. Willmer (1983) argues that while endotherms forage to maximise rewards, ectotherms forage to minimise costs.

I suggest that increased time spent on foraging may entail a number of costs and that these may differ for males and females. Increased chance of predation while foraging is a potential cost for both sexes. The observation that butterflies tended to forage with wings closed (i.e. less apparent than with wings open) supports this view. Other potential costs associated with foraging may be thermoregulatory e.g. cooling below optimum body temperature, or the risk of overheating at high SR intensity. For mated females, harassment by males while feeding may be another potential problem. For males, there may be a conflict between allocating time to feeding and time to mate location.

* No difference in duration of feeding bouts between "same" and "different" flower species was found here, but this could be due to foraging not being broken down into its component parts - e.g. the time taken for the proboscis to probe a flower before the nectaries are located.

CHAPTER 11. EGG-LAYING

11.1 Introduction

Realised fecundity (the complement of eggs laid in an individual's life time) is an important measure of individual success in female butterflies (Kingsolver, 1985a). Realised fecundity depends on a range of factors such as; length of time of female survival, adequate food and resources for egg development within the female (Porter 1992), suitable weather conditions for flight required to locate egg-laying sites and suitable weather conditions for egg-laying (Kingsolver 1983a, 1983b).

In other species, factors which influence rate of egg production include: temperature and nutrient intake (Stern & Smith 1960, Boggs 1986) female size and age (Stern & Smith 1960, Boggs 1986, Wickman & Karlsson 1987). In female meadow browns, most of the development and maturation of eggs occurs after emergence from the pupa. Eggs are laid singly, on a wide variety of fine leafed grasses (Pollard 1981, Brakefield 1982a, Feber 1983). Wiklund (1984) reports that eggs are frequently laid on dead plant material, non-plant material or "dropped" (not glued on to any substrate).

In this study I examine proximal factors related to egg-laying; behaviour of females, substrates used, weather conditions associated with egg-laying (and which might limit the number of eggs laid), number of eggs laid and percent of activity time allocated to egg-laying. I ask whether egg-laying behaviour differs between southern and northern populations and whether such differences influence potential realised fecundity.

11.2 Methods

Egg-laying was observed when following individual female butterflies. The time, duration and environmental variables associated with egg-laying behaviour were recorded following the protocols described in Chapter 3, section 3.2.

11.3 Results

Eight females were seen laying eggs in the southern population (laying a total of 77 eggs) and seven females in the northern population (27 eggs laid). Egg-laying was only observed in short grass areas in both regions.

11.3.1 Behaviour

A similar pattern of behaviour was observed for females in northern and southern populations. The pattern of behaviour, from the initial searching flight until a new behaviour is adopted, is here termed the "egg-laying sequence". The typical egg-laying sequence is as follows: The female would engage in a slow, fluttering flight (searching flight), low over the grass. She would then alight, low in the grass, and walk (with intermittent fluttering) with wings either closed (ventral eye spot half covered) or wings opening and closing. When a suitable place was found she would stop and curl her abdomen round, placing an egg low down onto a grass stem or leaf blade, usually within 1 cm of the ground. She would then walk/flutter a short distance (mean = 0.72 m ± 0.10, n = 42) and lay another egg. This behaviour (walk/flutter - egg) was usually repeated several times. The sequence could also include further short duration, low, searching flights (Figure 32). The egg-laying sequence was considered finished when the female started a new behaviour (e.g. fast flying, feeding, basking etc.).

11.3.2 Ambient air temperature and solar radiation associated with egg-laying

In both regions egg-laying behaviour took place over a wide range of ambient air temperatures and solar radiation levels. In the south, egg-laying was observed with T_a ranging from 16.3°C to 22.9°C and SR from 188.4 Wm^{-2} to 833.4 Wm^{-2}. In the north, T_a during egg-laying ranged from 15.1°C to 23.6°C and SR ranged from 160.0 Wm^{-2} to 951.5 Wm^{-2}. The mean ambient air temperature at which northern females laid their eggs was 2.4°C cooler than T_a for southern females (18.4°C and 20.8°C respectively). This difference is significant ($t = 3.92$, $df = 102$, $P < 0.001$). The mean solar radiation level at which northern females laid their eggs is also significantly lower than for southern females (470.3 Wm^{-2} and 664.2 Wm^{-2} respectively; $t = 3.45$, $df = 102$, $P = 0.001$).

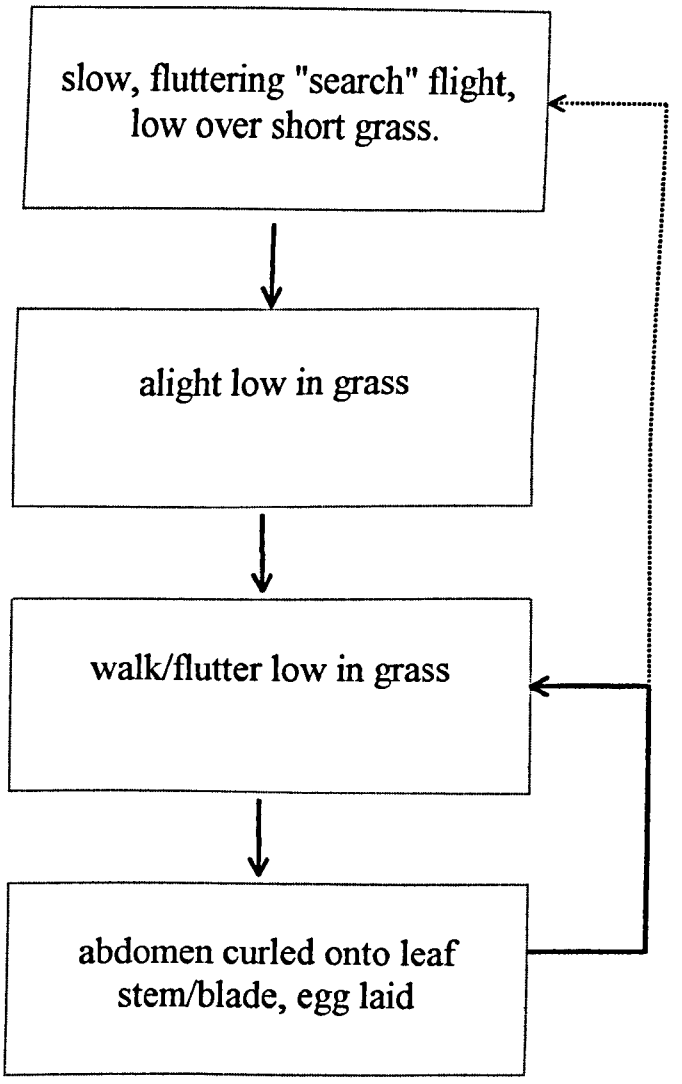


Figure 32. Egg-laying sequence, *Maniola jurtina*.

In both regions eggs were laid at ambient temperatures which were significantly lower than the overall mean for the region (southern population: $t = 3.21$, $df = 217$, $P = 0.001$; northern population: $t = 3.92$, $df = 39$, $P < 0.001$). In the southern population the solar radiation levels at which eggs were laid were significantly higher than the overall mean for the region ($t = 4.64$, $df = 217$, $P < 0.001$), but for the northern population there is no significant difference between the SR levels at which eggs were laid and the overall mean for the region.

One of the limitations of this study is that there were no direct measurements of ground temperature at the sites of egg laying. A number of studies have shown that microclimate is an important factor influencing choice of egg laying sites (reviewed in Porter 1992), so ground temperature at the site of egg laying may have a greater influence on oviposition behaviour that ambient air temperature.

11.3.3 Duration of egg-laying sequence and number of eggs laid

Summary statistics for the components of egg-laying behaviour are shown in Table 22. The mean duration of the whole egg-laying sequence was shorter for northern than for southern females, and northern females laid fewer eggs per sequence than did southern females. Neither of these differences is statistically significant. Females in both regions took a mean of approximately 16 seconds to lay an individual egg. The mean duration of walking between laying individual eggs was significantly longer for northern females than southern females. Dividing the sequence duration by the number of eggs laid during the sequence gives a value for the rate of egg-laying (seconds egg^{-1}) this is then converted to eggs laid min^{-1}. During the egg-laying sequence of behaviour, females from the southern population laid eggs at a greater mean rate than northern females (Table 22).

Table 22. Mean duration (s) of egg-laying sequence and its component behaviours, with mean number of eggs and frequency of egg-laying (eggs min^{-1})

	SOUTHERN ♀	NORTHERN ♀	Mann-Whitney U
duration of sequence (s)	326 .2 ± 55.9 (n = 8)	223.4 ± 77.5 (n = 7)	NS
duration of walk/flutter between eggs (s)	10.9 ± 1.0 (n = 88)	19.1 ± 2.0 (n = 62)	z = 2.90 p = **
duration of laying individual egg (s)	16.7 ± 1.2 (n = 77)	16.0 ± 1.9 (n = 27)	NS
no. eggs per sequence	9.6 ± 2.9 (n = 8)	3.8 ± 1.2 (n = 7)	NS
frequency of egg-laying (eggs $minute^{-1}$)	1.8 ± 0.3 (n = 8)	1.3 ± 0.3 (n = 7)	z = 2.89 p = **

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$, NS = not significant

11.3.4 Percent of time allocated to egg-laying behaviour

For the individuals which were observed laying eggs, the egg-laying sequences represent a mean of 29% of total contact time (time spent observing a particular individual) for southern females and 17% of total contact time for northern females. This difference is not significant (data arcsine transformed to approximate normality). Overall, egg-laying behaviour accounted for 10% of the time spent observing all southern females and 4% of the time for northern females. The overall trend is for northern females to lay fewer eggs during each egg-laying sequence, to lay eggs at a slower rate and to allocate less time to egg-laying behaviour.

11.3.5 Environmental variables and duration of egg-laying

Data for individual eggs laid (from when the female is alighted and begins to curl her abdomen into position until she removes her abdomen from the grass blade and moves on) are used to investigate the influence of T_a and SR on the duration of time taken to lay an egg. The influence of T_a and SR on the time taken to walk/flutter between each egg laid is also investigated. (Durations are log-transformed to give an approximation

to normality). For northern females regression analysis shows no significant relationship between either T_a or SR and the duration of egg-laying or walking, although there was a tendency for the duration of egg-laying to decrease with increasing solar radiation. For southern females the duration of egg-laying and walking is not related to T_a, but the duration of both egg-laying and walking decreases with increasing SR (egg-laying duration: $r^2 = 0.16$, df = 76, $P < 0.001$; walking/fluttering duration: $r^2 = 0.09$, df = 87, $P = 0.004$), Figure 33.

Stepwise multiple regression was carried out to determine whether other environmental variables (T_a, ground temperature and wind) had any additional influence on the variation in duration, but only SR remained significant.

11.3.6 A model for egg production in *M.jurtina*

A model is developed here to estimate the difference in realised fecundity for females in the southern and northern populations. The information and assumptions used in this model are based on the work of Gossard & Jones (1977), Dowdeswell (1981), Brakefield (1982b), and Kingsolver (1983a, 1985a).

The model uses the assumption that egg-laying behaviour is limited by time available for active flight (Kingsolver 1983a) and that time available for active flight is limited by weather conditions (T_a and SR). Here I make the assumption that time available for active flight equates with mean hours of sunshine day^{-1}, (but that this time will also include other behaviours such as feeding and basking, which are interspersed with flight). Information used in the model includes: the number of eggs which females can lay in captivity (250 eggs, Dowdeswell, 1981, assumed to be the maximum potential egg production) and the average lifespan of females (6.5 days, but with oviposition beginning on the second day after eclosion, making the "egg-laying, or fecund lifespan" 5.5 days, Brakefield 1982b). I also use estimates on the decline in egg production with age for *P. rapae* (Gossard & Jones 1977). The percent of time allocated to egg-laying and the rate of egg production differed for females in the southern and northern regions, so this is incorporated into the model.

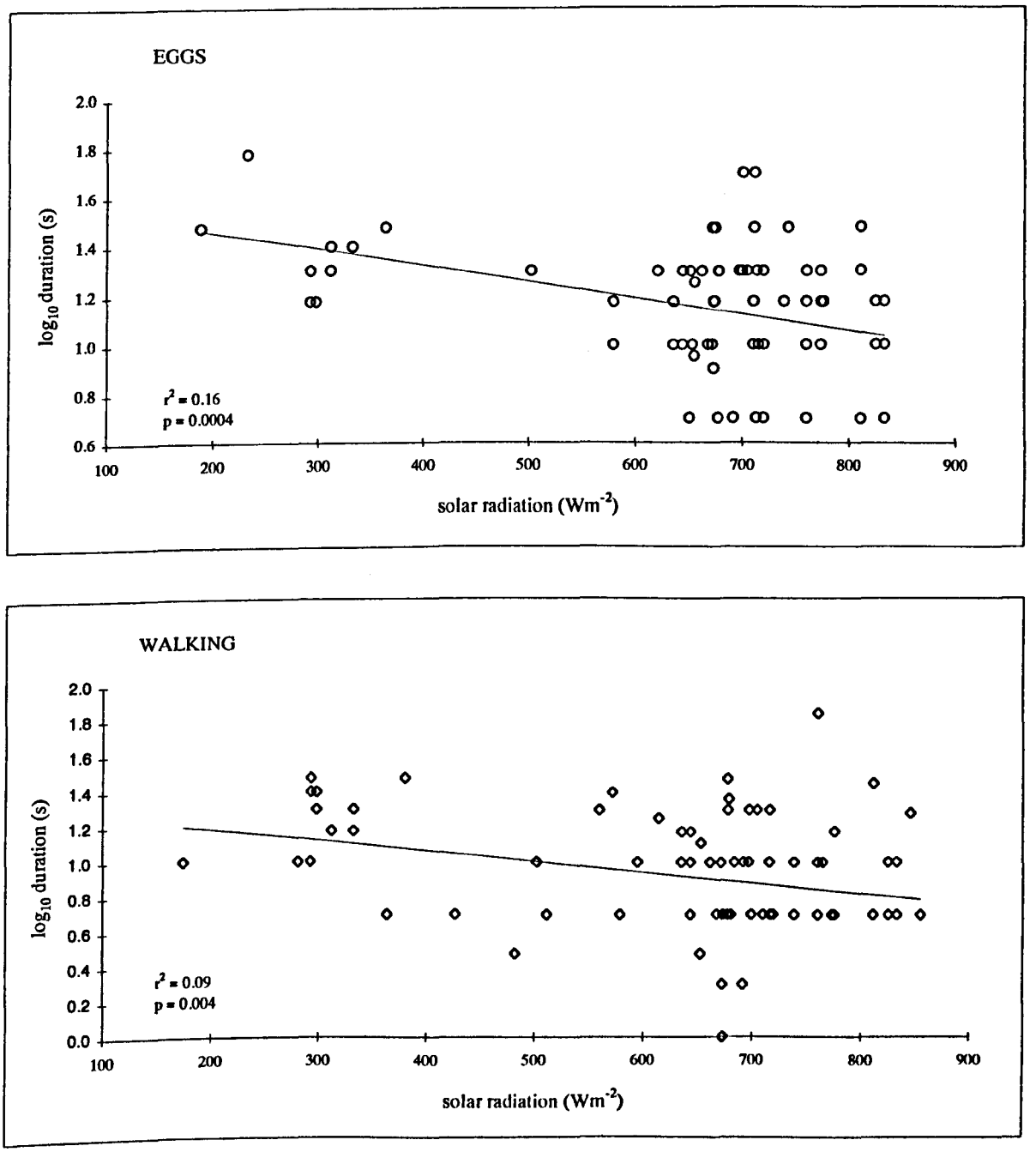


Figure 33. Duration of laying individual eggs and duration of walking between laying eggs, in relation to solar radiation intensity. *Maniola jurtina* , southern population.

The Model

In the southern population the mean hours of daily sunshine for July is 6.1 (Chapter 4). If the mean fecund life span of a female is 5.5 days, then this give an estimate of 33.5 hours (2010 minutes) of activity over the female's fecund life. At the population level, 10% of time was allocated to egg-laying activity (egg-laying sequences of behaviour). This gives an estimate of 201 minutes (12060 s) of time allocated to egg-laying sequences. In the southern population the mean duration of each laying sequence was 326 s. This gives an estimate of 37 egg-laying sequences over the female's fecund life, or 6.7 sequences day^{-1}. The mean number of eggs laid for each sequence was 9.6, which gives an estimate of 64 eggs day^{-1}. If egg production was uniform at this rate, it would give an estimate of 352 eggs over the female's lifetime. Brakefield (1982b) found that peak egg production occurred at 2 -5 days after mating, and then steadily declined. As 64 eggs day^{-1} appears to be a high rate of egg production, this is taken to be the peak rate, after which it declines on a daily basis. Gossard & Jones (1977) found that egg production in *P. rapae* decreased with age. They calculated that the rate of daily reduction in unlaid eggs was 0.78. Using this figure, I assume that on day 0, there were 250 unlaid eggs (the figure for eggs laid by butterflies in captivity). On day one, 64 eggs were laid (186 unlaid eggs). The number of unlaid eggs then decreases by 0.78 each successive day, so on day 2 there are 186 x 0.78 = 145 unlaid eggs (41 eggs laid), on day 3 there are 145 x 0.78 = 113 unlaid eggs (32 eggs laid) etc. By 5.5 days a total of 189 eggs have been laid, which represents 76% of potential egg production. The mean density of southern females was 1.29 butterflies for each 100 m (transect data). This gives an estimate of eggs laid of 189 x 1.29, i.e. 244 eggs hm^{-1}.

The same calculations are repeated for butterflies in the northern population, with changes in the following variables: Mean hours of sunshine day^{-1} = 4.3; the percent of time allocated to egg-laying (at the population level) = 4%; mean duration of egg-laying sequence = 223.4 s; mean number of eggs laid for each sequence = 3.8. Using these figures the estimated egg production for northern females, over their life time is 149

eggs, which represents 60% of potential egg production. The mean density of northern females was 0.19 butterflies hm^{-1}, giving an estimate of 28 eggs hm^{-1}*.

This model is intended to give an indication of the potential difference in realised fecundity between the southern and northern populations, rather than an accurate estimate of actual egg production. The reason that the latter is not possible here is the number of variables not included (e.g. temperature, age of females, etc.) and the untested nature of some of the assumptions (e.g. rate of decrease in unlaid eggs).

11.4 Discussion

Kingsolver (1985a) argues that weather (principally temperature and solar radiation) influences population dynamics of butterflies through its effect on realised fecundity, with time available for oviposition being restricted to conditions compatible with active flight. In the northern region, weather conditions are generally less favourable for activity (lower temperatures, fewer hours of sunshine) so a lower level of egg production is expected. Although in this study northern and southern females spent a similar proportion of their time in active flight, northern females allocated a smaller proportion of their time to egg-laying behaviour. The rate of oviposition also decreased with lower solar radiation intensity (duration time taken to lay each egg and time taken to walk between laying eggs increased - data for southern females). The lower temperatures in the northern region are also likely to decrease the rate of egg maturation and ovipositions (Stern & Smith 1960).

The model presented here indicates that realised fecundity of northern females is lower than that of southern females. Brakefield (1982b) estimated that female *M. jurtina* in Hightown (near Liverpool) laid only 37% of their potential egg production, whereas those in the Scilly Isles laid 46%. Kingsolver (1983b) estimated that *Colias* living at high elevations lay between 20% and 50% of their potential egg production, whereas those at low elevation lay up to 72%. Although the values differ, the pattern to emerge

*The model gives "egg densities" in eggs hm^{-1} (number of eggs for each 100 m, using recorded transect density of female meadow browns). Although this is a linear measurement, butterflies on transects were recorded if they occurred within approximately 2.5 m either side of the transect route, so the measure could also be considered as eggs 500 m^{-2}.

is the same as that presented here - that in less favourable conditions (higher latitude or elevation) the realised fecundity of females is reduced.

This supports the view that the weather in different geographic areas effects population dynamics through its influence on realised fecundity. Here, the lower rate of egg production in northern females is one of the factors contributing to the low population density of *M. jurtina* in the northern region. At the population level, fewer eggs are laid, and adverse weather conditions also may lead to increased mortality of larvae through predation and parasitism (Pollard 1979, Warren *et al.* 1986, Warren 1992) further decreasing the density of northern meadow browns.

CHAPTER 12. THORACIC TEMPERATURES

12.1 Introduction

In temperate regions butterflies usually need to raise their body temperature several degrees above that of ambient air temperature before the flight muscles of the thorax are warm enough for active flight (Heinrich 1993). Most butterflies warm-up prior to flight by choice of suitable microhabitat and substrate (Casey 1981, Shreeve 1984, Rutowski *et al.* 1994) and by basking to absorb solar radiation (Clench 1966, Wasserthal 1975, Watt 1968, 1969, Douglas & Grula 1978, Kingsolver 1983a, 1983b). Thoracic temperatures required for flight initiation, for temperate species, have been found in the range of 28 - 34°C. Examples of T_{th} for flight initiation are: 30°C for *Colias nastes* (Roland 1982), 32-34° C for *Pararge aegeria* (Shreeve 1984), 28-30° C for *Papilio machaon* (Wasserthal 1975) and 29-30° C for *Pieris* spp. (Kingsolver 1985c).

During flight T_{th} may remain stable (Tsuji *et al.* 1986), decrease (Shreeve 1984, Shelly & Ludwig 1985, Heinrich 1986a, 1986b,) or increase (Kammer 1970, Rawlins 1980). Which of these options actually occurs will depend on the species, the environmental conditions and the wing stroke frequency and activity level. The general pattern is for small and medium sized butterflies to loose heat during flight, by convective cooling, whereas larger butterflies are able to maintain a higher steady state body temperature during flight (Heinrich 1986b). At high solar radiation intensity heat avoiding strategies may be adopted and flight activity may be limited by the dangers of overheating (at $T_{th} \geq 40$°C, for *Colias,* Kingsolver 1983a).

In marginal conditions with lower ambient temperatures and fewer hours of sunshine, which limits time available for flight, butterflies might be expected to show adaptations which enable them to warm up more rapidly, reach the necessary T_{th} at lower T_a and SR intensities, or fly at lower T_{th} (or a combination of these).

In this project a number of questions are asked about thoracic temperatures of *M. jurtina*:

1. What are the thoracic temperatures associated with different behaviours, such as escape flight, basking, slow flying/feeding, and active flight?
2. How do environmental variables such as T_a and SR influence T_{th}?
3. Do northern and southern populations show any differences in the T_{th} associated with active flight?

12.2 Methods

Thoracic temperatures of butterflies in the field were recorded by using the "grab and stab" technique, described in Chapter 3, section 3.3. A total of 48 butterflies were caught from the southern population in both July 1994 and July 1996. These butterflies were engaged in a range of behaviours immediately prior to capture, classified as "flew when disturbed" (FWD - escape flight), slow flying/feeding (butterflies feeding and fluttering between flower heads), active flight (butterflies flying vigorously) and basking. 16 butterflies were caught from the northern population in July 1995, all had been engaged in active flight prior to capture. Data for male and female butterflies are combined.

12.3 Results

12.3.1 Thoracic temperatures associated with different behaviours

Using data from the southern population, the highest thoracic temperatures were associated with active flight (28 - 35°C, $\bar{x}$ = 31.0 °C) and lowest with FWD (20 - 29°C, $\bar{x}$ = 23.0°C). Thoracic temperatures associated with slow flying and basking were intermediate between these two. The differences between the mean T_{th} for each behaviour are all significant with exception of slow flying and basking* (Table 23).

* multiple t-tests are used rather than ANOVA, as uneven numbers for each behaviour disallow the latter.

Table 23. Mean T_{th} (°C) associated with different behaviours with significance of differences in T_{th} (two-tailed t-test, assuming unequal variance). *M.jurtina*, southern population.

Activity	*Mean T_{th} ± s.e.*	*n*	*Comparison*	*t*	*P*
FWD	23.0 ± 1.2	8	FWD - slow flight	2.24	*
slow flight	25.9 ± 0.7	15	FWD - basking	2.15	*
basking	28.2 ± 2.5	4	FWD - active flight	8.88	***
active flight	31.0 ± 0.3	21	slow flight - basking	-	NS
			slow flight - active flight	7.56	***
			active flight - basking	2.25	*

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
NS = not significant

12.3.2 Thoracic temperature and environmental variables

May & Casey (1983) suggest that if T_{th} is plotted against T_a, and the best-fit line of regression has a slope of less than 1, there is evidence for thermoregulation. When T_{th} is plotted against T_a at the time of capture, there is a significant correlation, with the best-fit regression line showing a slope of less than 1 (Figure 34a.) This indicates that the butterflies are able to thermoregulate to control their thoracic temperature. Only 12% of the variation in T_{th} is explained by variation in T_a.

T_{th} also shows a significant relationship with solar radiation levels at the time of capture, with a polynomial (order 3) best-fit curve, and a much higher percent of the variation in T_{th} (70%) explained (Figure 34b).

The influence of solar radiation on thoracic temperature can be demonstrated in an additional way, by plotting temperature excess ($T_{ex} = T_{th}-T_a$) against solar radiation. At very low SR intensity (SR ≅ 100 Wm^{-2}) T_{th} is only 1 - 2°C above ambient. The greatest T_{ex} is at high SR intensity ($\geq$ 800 Wm^{-2}) when T_{th} is 14 - 16°C above ambient air temperature (Figure 34c).

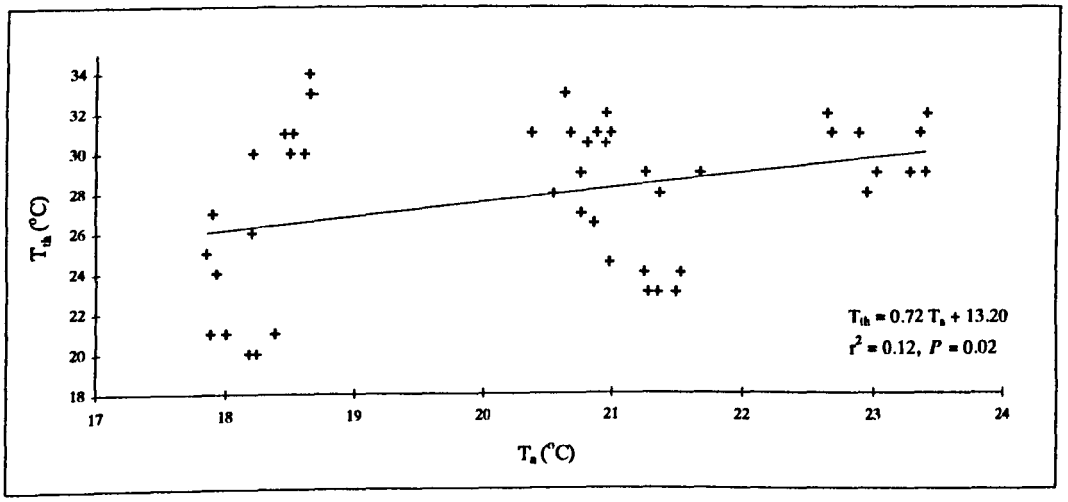


Figure 34a. Thoracic temperature (T_{th}) in relation to ambient air temperature (T_a).
Maniola jurtina, southern population.

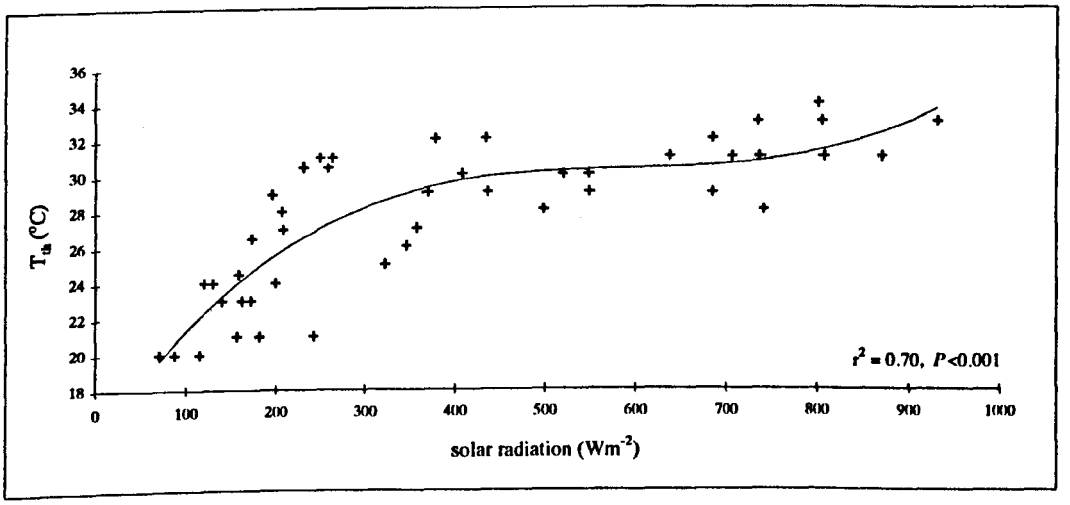


Figure 34b. Thoracic temperature (T_{th}) in relation to solar radiation (SR).
Maniola jurtina, southern population.

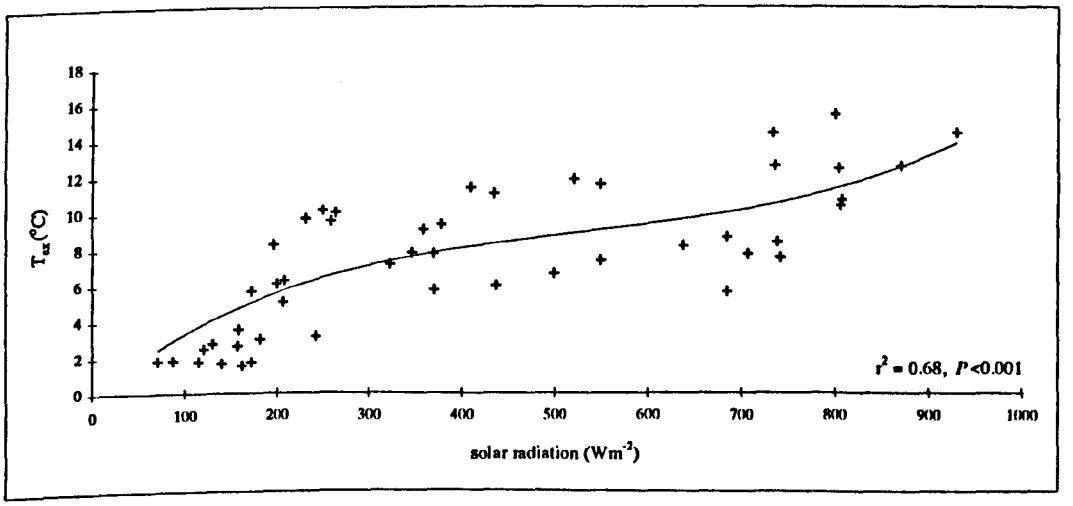


Figure 34c. Thoracic temperature excess (T_{ex}) in relation to solar radiation.
M. jurtina, southern population. ($T_{ex} = T_{th} - T_a$)

If T_{th} is plotted against ground temperature at the time of capture, and a best-fit polynomial regression line is fitted, then 76% of the variation in T_{th} is now accounted for (Figure 35a). The shape of the best-fit line shows that at ground temperatures of between 19°C and 23°C, T_{th} increases in an approximately linear fashion in relation to ground temperature, with thoracic temperatures about 3°C above that of ground temperature. Between ground temperatures of approximately 24°C and 32°C, T_{th} remains fairly constant, within the range of 28°C to 33°C (mean ≅ 30°C).

The high percent of variation in T_{th} which is explained by variation in ground temperature, is probably due to two factors. The first is that SR and T3 are strongly correlated (Chapter 4), so variation in ground temperature correlates with the amount of solar radiation being absorbed by the butterfly. The second is that substrates on the ground or low in the vegetation provide warm, sheltered microclimates, which may help to increase thoracic temperatures beyond the influence of SR alone (Casey 1981, Shreeve 1990, Rutowski *et al.* 1994).

When the behaviour associated with each T_{th} data point is identified (Figure 35b), then it becomes apparent that T_{th} or slow flying/feeding usually falls below the best-fit line (i.e. lower than predicted by the regression equation). Two options (not mutually exclusive) could account for this. The first is that during slow flight, thoracic temperatures fall to a greater extent than during active flight. The second is that thoracic temperatures are too low for active flight (butterflies cannot gain sufficient heat from basking) and only slow flight/feeding is possible.

The residuals of the polynomial regression of T_{th} against ground temperature (T3) are further regressed against the remaining environmental variables (T_a, air temperature at vegetation height (T2), SR and wind speed). None of these variables are significant when regressed against the residuals of T_{th} on T3, but there is a tendency for T_{th} to be higher than predicted at low wind speed, and lower than predicted at high wind speed (Figure 35c).

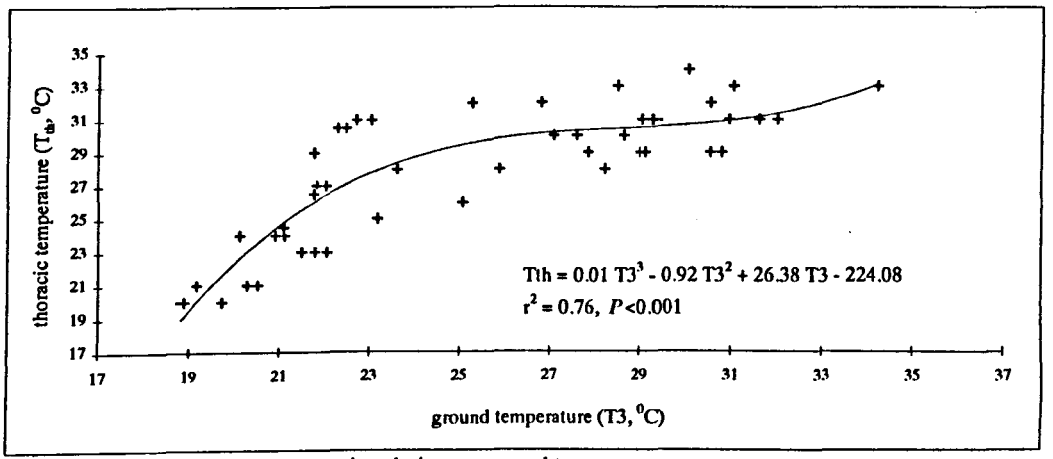


Figure 35a. Thoracic temperature in relation to ground temperature.

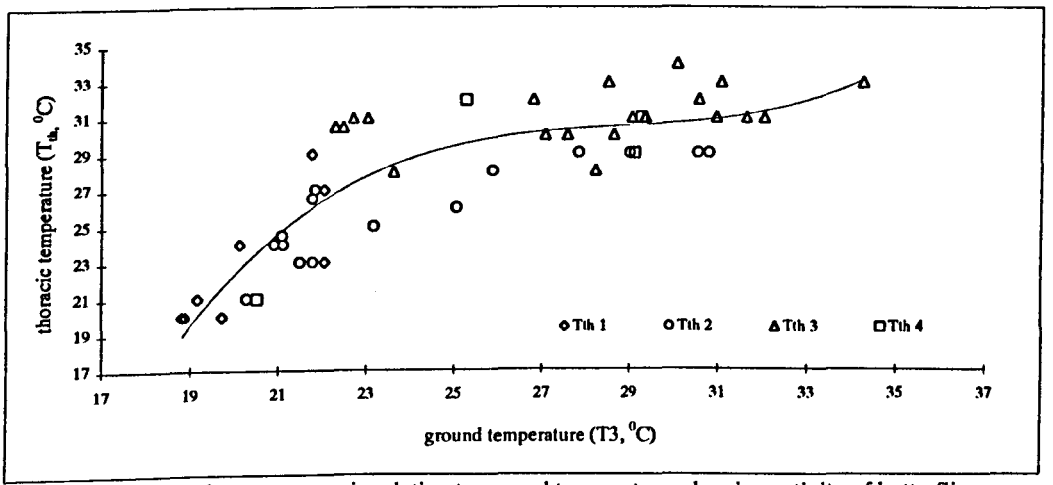


Figure 35b. Thoracic temperature in relation to ground temperature, showing activity of butterflies. Tth 1 = flew when disturbed, Tth 2 = slow flying/feeding, Tth 3 = active flight, Tth 4 = basking

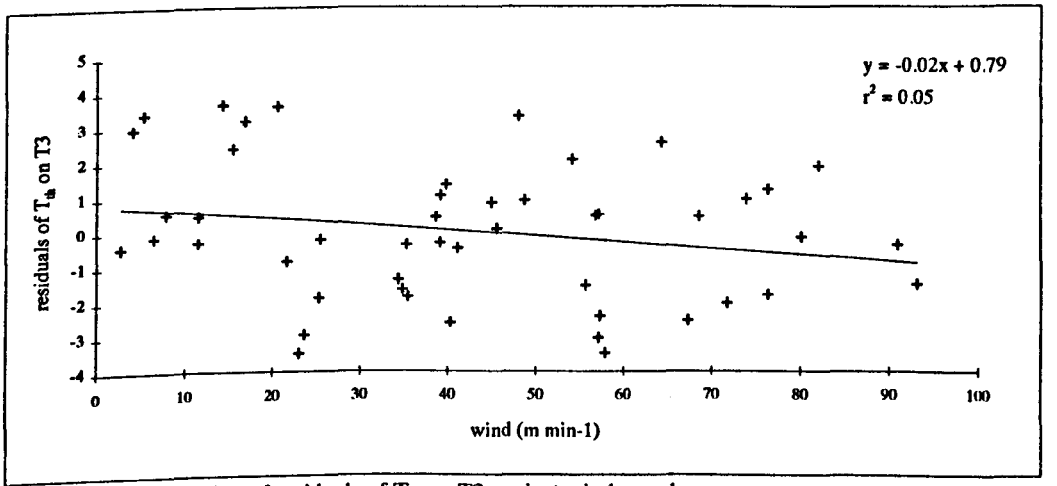


Figure35c. Regression of residuals of T_{th} on T3 against wind speed.

Figure 35. Thoracic temperature in relation to ground temperature and wind speed *Maniola jurtina* , southern population.

Thus variation in ground temperature and wind speed together account for 81% of the variation in thoracic temperature.

12.3.3 Thoracic temperatures and active flight in southern and northern regions

Thoracic temperature of butterflies engaged in active flight immediately prior to capture (17 individuals from the southern population and 16 from the northern population) are compared to test whether butterflies from the northern population fly at lower T_{th} than those from the south. The mean T_{th} for southern butterflies engaged in active flight was 31.1 ± 2.7°C, compared to 32.0 ± 4.3°C for northern butterflies. There is no significant difference between these two values.

12.4 Discussion

Errors associated with grab and stab

The grab and stab technique, although widely used, entails a number of assumptions about the level of accuracy of T_{th} obtained in this way. It is generally accepted that between the time of capture and insertion of the temperature probe, the butterfly will undergo passive cooling, so the recorded T_{th} will under-estimate the actual T_{th}. To minimise this error, the time between capture and recording the T_{th} is kept as short as possible. The "acceptable" time lag ranges from 5 s (Heinrich 1993) to 20 s (Guppy 1986b). The time lag in this study was ≅ 15 s. Within this time scale the decrease in thoracic temperature has been found to be ≤ 1°C (Rawlins 1980, May & Casey 1983, Heinrich 1986b). Stone & Willmer (1989) also point out that errors associated with passive cooling are not uniform over a range of ambient air temperatures. Critiques of the grab and stab technique include Stone & Willmer (1989) and Heinrich (1993).

Thoracic temperatures associated with different activities

For sustained vigorous flight to occur, the flight muscles must reach a minimum temperature of approximately 30°C. Below this temperature, short escape flight may be possible, but if thoracic temperatures are below a critical threshold, no flight is possible. *M. jurtina* could undertake short escape flights (FWD) with a mean thoracic temperature of 23°C (minimum 20°C), but for vigorous flight mean thoracic temperature was 31°C (range 28 - 35°C). Heinrich (1986b) found that *A. glandon*, a small butterfly, could fly

if disturbed with thoracic temperature of 20°C, but voluntary flight initiation occurred when T_{th} was between 32.8 and 34.3°C. The larger *Papilio polyxenes* could undertake short flights at T_{th} of 24°C, but vigorous flight did not occur until T_{th} reached 28°C (Rawlins 1980). The T_{th} recorded here for active flight in *M. jurtina* is similar to that recorded for flight initiation and active flight for other species: 30 - 40°C for *Colias* (Kingsolver 1983a, 1983b, Kingsolver & Watt 1983), 30° C for *Colias nastes* (Roland 1982), 32-34° C for *Pararge aegeria* (Shreeve 1984), 28-30° C for *Papilio machaon* (Wasserthal 1975) and 29 - 30° C for *Pieris* spp. (Kingsolver 1985c).

Thoracic temperature recorded for active flight will vary depending on the amount of time for which the butterfly has been flying. For small and medium sized butterflies, temperatures will be highest at flight initiation but will usually have fallen by several degrees by the time the butterfly lands. Shreeve (1984) found *P. aegeria* cooled by a mean of 6.5 °C during flight. Heinrich (1986b) recorded T_{th} of 32.8 - 34.3°C at flight initiation, after basking, for *A. glandon*, with a cooling to 25.1 - 25.6°C on landing. Similarly, *C. inornata* initiated flight with a mean T_{th} of 31°C after basking, and had cooled to 25.3°C on landing. Here, thoracic temperatures taken when *M. jurtina* had previously been engaged in active flight varied between individuals by up to 6°C for any given T_a, presumably reflecting the amount of time (and hence degree of cooling) that the individual had been engaged in flying.

Thoracic temperatures and environmental variables

May & Casey (1983) argue that when T_{th} is plotted against T_a and the slope is < 1, this provides evidence of thermoregulatory behaviour. They used this argument for endothermic euglossine bees, which thermoregulate by shivering to generate heat at low T_a, and shunting heat (via the haemolymph) between the thorax and abdomen at higher T_a and T_{th}. The butterfly *P. polyxenes* also generates heat by shivering when solar radiation intensity and ambient air temperatures are low. At higher SR intensity it basks to absorb solar radiation and can adjust thoracic temperature by altering the abdominal posture - raising the abdomen to decrease haemolymph flow and heat transfer from thorax to abdomen at low SR intensity, and lowering the abdomen to increase haemolymph flow and heat transfer at high SR intensity (Rawlins 1980).

Most butterflies, including meadow browns, thermoregulate by behavioural means, adjusting wing angle and orientation in relation to incoming solar radiation, and by alternating between flying (T_{th} decreases) and basking (to increase T_{th}). Despite the difference in means of thermoregulation between endotherms and ectotherms, the argument proposed by May & Casey still applies; that is that variation in thoracic temperature is less than would be predicted by variation in T_a if no thermoregulation was taking place. In the case of butterflies, where solar radiation is crucial for raising thoracic temperature, one can also argue that variation in T_{th} should be less than expected by variation in SR.

The pattern found here for *M. jurtina* supports this argument. When T_{th} is plotted against SR or ground temperature (T3), at low SR or T3 thoracic temperature increases, before levelling out and remaining fairly stable as T3 or SR increase (i.e. varying less than would be expected for a non-thermoregulating animal). T_{th} then increasing again at high SR or T3, but at a temperature below that of a hypothetical non-thermoregulating animal (which would show a linear increase in temperature with a slope of 1). Although the plot here is for T_{th} of different individuals at different SR or T3, it is assumed that a continuous plot of T_{th} for one individual should show the same form as the animal switched from basking to intermediate (graded) to heat avoiding behaviour. The pattern found here is the same as that described by Dreisig (1995) for a hypothetical thermoregulating ectotherm in the field. Rawlins (1980) found a similar pattern of variation in T_{th} in relation to T_a for *P. polyxenes*.

The importance of SR for elevating thoracic temperatures is also demonstrated by the relationship between temperature excess (T_{ex}, the difference between T_{th} and T_a) and SR intensity. The highest T_{ex} for *M. jurtina* was recorded at high SR intensity, with T_{th} up to 15°C above T_a, at SR intensities >700 Wm^{-2}, while at low SR intensity (<150 Wm^{-2}) T_{th} was only 1 - 2°C above ambient.

Thoracic temperatures of Southern and Northern butterflies

No significant difference was found between southern and northern populations in the mean T_{th} for active flight. This suggests that northern butterflies are not adapted to living in more marginal conditions by being able to fly with lower T_{th} than southern butterflies. This conforms to the pattern found for other species with populations living in different climatic zones. Heinrich (1986b) found that there was no difference in the range of T_{th} for active flight for low and high altitude populations of four species of butterflies. Kingsolver & Watt (1983) also found no difference in the T_{th} for flight initiation of *Colias* populations living at high or low altitude, or in the maximum body temperatures (reflecting the upper limit of physiological tolerance) for high and low altitude populations. They did, however, find that mean T_{th} for basking was lower at high elevations and that high elevation *Colias* experienced a greater variation in body temperature than those at low elevations, reflecting the greater variation in meteorological conditions.

The finding that southern and northern populations of *M. jurtina* fly at the same T_{th} suggests that both populations have similar physiological thresholds for active flight. The following chapter explores whether there are any morphological adaptations which increase the probability of individuals achieving the required T_{th} for active flight at lower T_a and SR intensities.

CHAPTER 13. THERMOREGULATION: WING MORPHOLOGY AND MELANIZATION

13.1 Introduction

A large body of work has confirmed the importance of wings in regulating butterfly body temperature (e.g. Watt 1968, 1969, Wasserthal 1975, Kingsolver 1985b, 1987a), with butterflies absorbing solar radiation to raise thoracic temperature (Chapter 2, section 2.2.3).

Several studies have investigated both seasonal and climatic (usually altitudinal) variation in melanization, and related this to an energy absorption advantage for the darker forms. Populations of *Colias* (lateral basking species) studied in N. America show increased melanization of the ventral wings (particularly hind wings) in cooler climates at both higher latitudes and altitudes (Watt 1968, 1969). *Parnassius phoebus*, a dorsal basking species, shows increased melanization of the dorsal wing surface at higher altitude and latitude (Guppy 1986a). *Colias* also show seasonal variation in melanism, with darker forms being more frequent at colder times of year (Watt 1969). Darker forms have been shown to heat up faster and maintain a higher steady state temperature than lighter forms (Watt 1968), allowing them to achieve thoracic temperatures required for flight under ambient temperatures which would be limiting for lighter forms (Watt 1969). Lighter forms may be maintained in the same population as under conditions of high solar radiation they would be able to remain flying for longer when darker forms would overheat.

Body size may also affect thermoregulatory efficiency and activity of butterflies. Smaller individuals warm up more rapidly than larger ones, but once warmed larger individuals are able to maintain a more stable body temperature (Shreeve 1992, Heinrich 1986b).

Previous research on meadow browns has found that variation in wing morphology has a seasonal component, with smaller individuals being found later in the season (Brakefield 1984). Although interesting in its own right, seasonal variation is not

addressed here, with all analyses being carried out on individuals caught at the peak of the flight season (July).

In this study a number of questions are addressed in relation to the influence of wing morphology and melanization on thermoregulatory efficiency and activity:

1. Are there any differences between northern and southern populations in wing size and degree of basal melanization?
2. Are butterflies of different size and darkness active under different environmental conditions?
3. Do smaller or darker butterflies warm-up faster under laboratory conditions?
4. Do warm-up rates under laboratory conditions support evidence from field data?

13.2 Methods

Analysis of wing morphology was carried out on a total of 70 butterflies (51 males and 19 females) from the southern population and 72 (49 males and 23 females) from the northern population, caught during July 1996. Data for environmental variables (T_a and SR) at the time of capture are available for butterflies from the southern population but not for those from the northern population. Butterflies from the southern population were all active immediately prior to capture and were caught as they were encountered, with no attempt being made to discriminate between males and females.

Methods used for the measurement of wing morphology and laboratory warm-up rates are described in Chapter 3, sections 3.4.1 and 3.4.2. The wing morphological variables used in this study are wing area and basal grey value (BGV). Body (thorax) size was not measured directly, but it is assumed that wing area correlates with thorax size (Goulson 1991). For warm-up rates, a mean of the increase in temperature per minute, over the first ten minutes of warming up, is used in the analysis.

13.3 Results

13.3.1 Repeatability of wing morphology data

To ensure that measurements of wing morphology were consistent between sessions, a repeatability test was conducted. Morphological measurements (wing perimeter, length,

breadth, area and basal grey value) were repeated for the same specimen, ten times over four consecutive days. The highest sample variance of these measurements is 0.57 (for wing area). The maximum deviation from the mean of the repeated measurements is 1.3 % (for BGV). This confirms that there is a high consistency in measurements between sessions.

13.3.2 Differences between southern and northern populations

Summary data of wing area and basal grey values are show in Table 24. As expected, females are significantly larger than males in both southern and northern regions. Northern males are significantly larger than southern males, but there is no significant difference in the size of females between the two regions.

Table 24. Wing area (mm^2) and basal grey values (BGV; 0 = black - 255 = white) of dorsal forewings of *M. jurtina* (mean ± s.e.), with significance of differences (wing area: t- test assuming unequal variance, BGV: Mann-Whitney U test).

	N	*wing area (mm^2)* *mean ± s.e.*	*basal grey value (0 - 255)* *mean ± s.e.*
♂ south	51	189.3 ± 2.2	61.2 ± 0.8
♀ south	19	221.2 ± 4.3	86.0 ± 3.0
♂ north	49	200.7 ± 2.1	56.4 ± 1.0
♀ north	23	226.5 ± 3.9	73.8 ± 2.1

	wing area		*basal grey value*	
comparison	*t*	*p*	*z*	*p*
♂ south - ♀ south	7.09	***	5.51	***
♂ north - ♀ north	6.27	***	5.81	***
♂ south - ♂ north	3.68	***	3.68	***
♀ south - ♀ north	-	NS	3.65	***

significance levels: * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $0.001 \geq P$
NS = not significant

The basal area of the dorsal forewing is significantly darker (lower BGVs) in males than in females in both regions. Butterflies from the northern population (males and females) are also significantly darker than those from the south.

There is no correlation between size and BGV for either sex in southern and northern populations.

13.3.4 Wing morphology and activity under different environmental conditions

Data from the southern population are used in this analysis.

Basal Grey Values

Evidence from other species suggests that darker butterflies are more efficient at absorbing solar radiation and are able to achieve the thoracic temperatures required for flight faster than paler butterflies, at low ambient air temperatures. If this applies to meadow browns, then it is predicted that on cooler days, or under conditions of low solar radiation, darker butterflies within the population are likely to be more active than paler ones. Thus butterflies caught at low ambient air temperatures should have lower basal grey values than those caught at high T_a (when overheating may be a potential problem).

This hypothesis is confirmed using data from the southern population. There is a strong correlation between BGV and T_a at the time of capture for both male ($r^2 = 0.26, P < 0.001$) and female ($r^2 = 0.53, P < 0.001$) butterflies (Figures 36a and 36b). Multiple regression was carried out, adding SR a second independent variable, but this did not add significantly to the r^2 value (SR and T_a are significantly related, $r^2 = 0.21, P = 0.01$). On the basis of the role of melanin in absorbing solar radiation, and contributing to thoracic warming (see chapter 2, section 2.2.4), one might expect a stronger correlation between BGV and SR (along with the correlation between BGV and T_a) at time of capture. Repeating these observations with a larger sample size might help to clarify this apparent anomaly. Combining data for male and female butterflies, there is a weak correlation between BGV and SR at the time of capture ($r^2 = 0.08, P = 0.02$) (Figure 36c). (Treating male and female data independently, neither reach statistical significance). This indicates that darker butterflies are more likely to be active on days with low solar radiation levels, but as data for males and females are combined, and males are usually darker than females (see above), then male butterflies are more likely to be active than females under low solar radiation conditions.

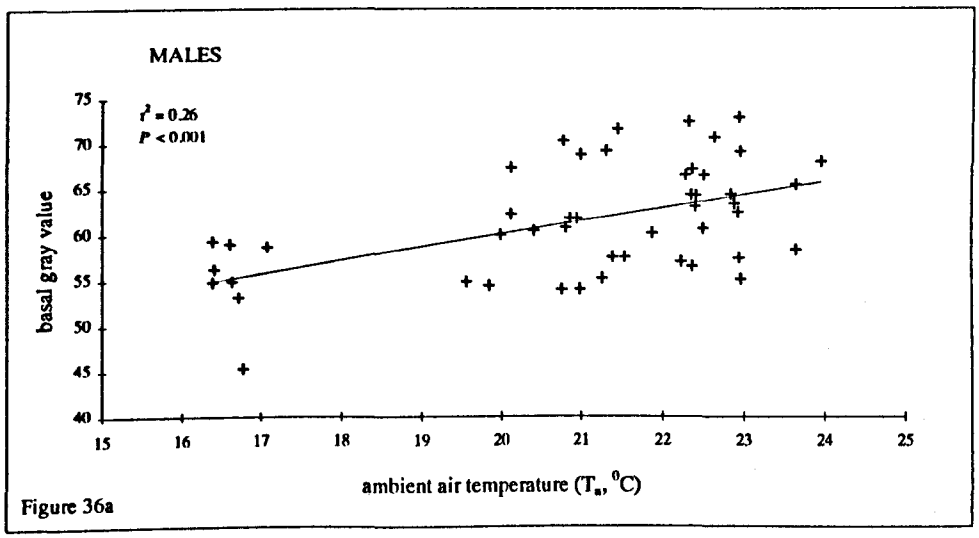


Figure 36a

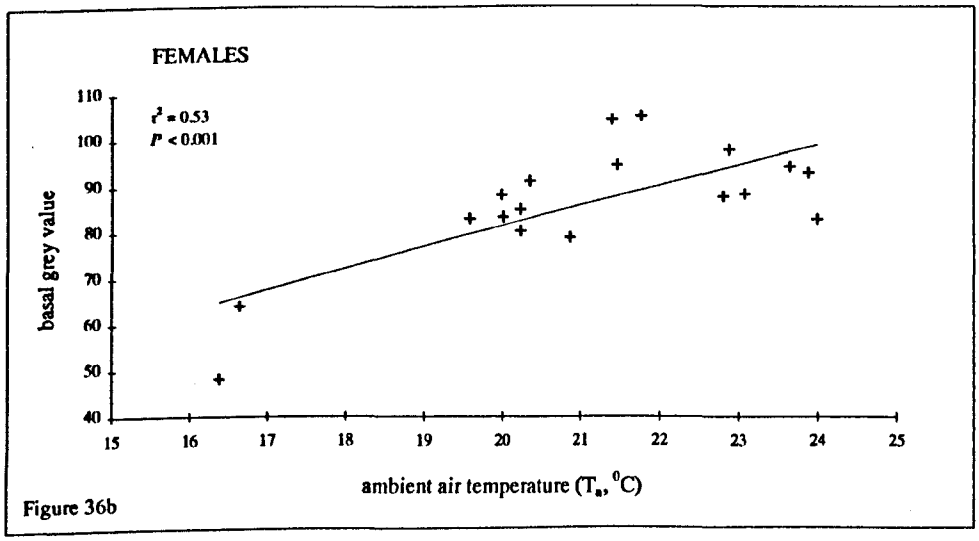


Figure 36b

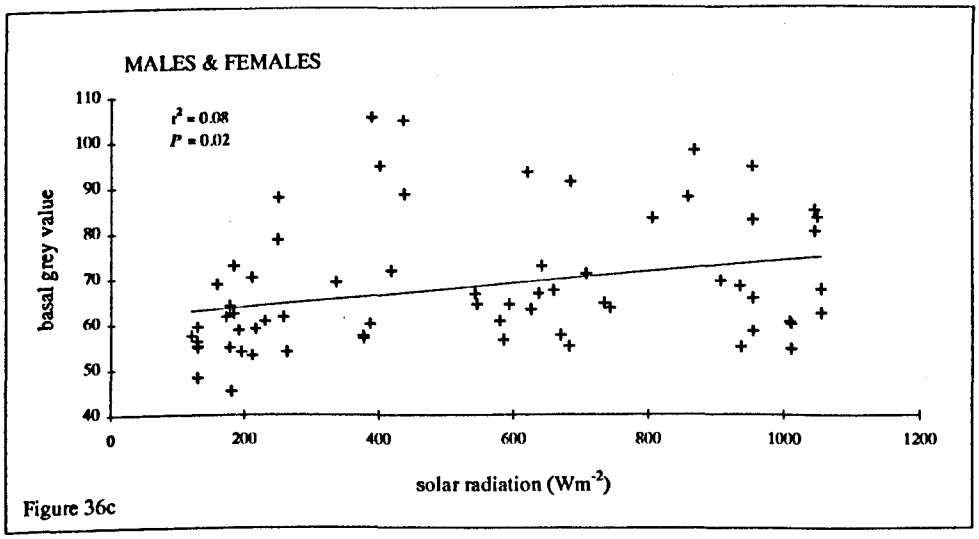


Figure 36c

Figure 36. Basal grey value of the doral forewing in relation to ambient air temperature and solar radiation when butterflies were captured. *Maniola jurtina*, southern population.

Wing Areas

Smaller butterflies of both sexes tended to be more active under low T_a, supporting the hypothesis that smaller butterflies warm up faster than larger butterflies, but the relationship between T_a and wing area is weak (males: $r^2 = 0.09$, $P = 0.04$, females: $r^2 = 0.20$, $P = 0.06$) (Figures 37a and 37b).

When data for the sexes is combined, there is a significant correlation between wing area and solar radiation levels at which the butterflies were caught ($r^2 = 0.12$, $P = 0.01$) (Figure 37c). As males tend to be smaller than females, this is again probably due to males being more active than females under low SR conditions.

Again, multiple regression using T_a and SR together had did not make a significant addition to the r^2 value.

Interaction between wing area and basal grey value

It is possible that there is an interaction between wing area and BGV in terms of SR intensity under which the butterflies are active. The data set is too small to split into categories of small-dark, small-paler, large-dark, large-paler, which would allow a statistical analysis of these morphological variants. Data can however be plotted as SR under which the butterflies were caught against wing area and BGV (Figure 38). This allows a visual interpretation of trends involving the interaction of wing area and BGV.

For male butterflies there is a clear trend for smaller darker butterflies to be active at low SR intensity. Both small-paler butterflies and darker-larger butterflies tend to be active at higher SR intensities. For females a similar trend is apparent, though less clear cut, with the main trend being for larger individuals to be active at higher solar radiation intensities.

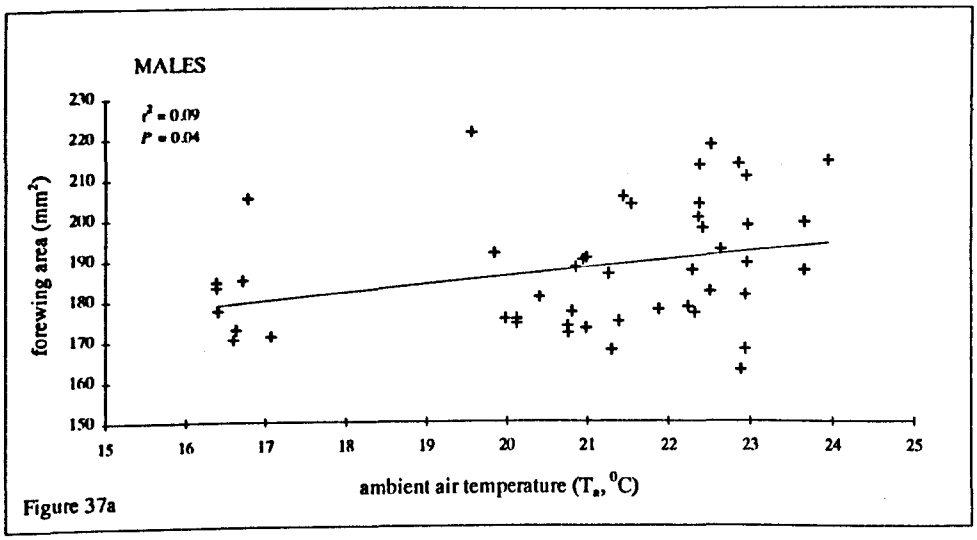


Figure 37a

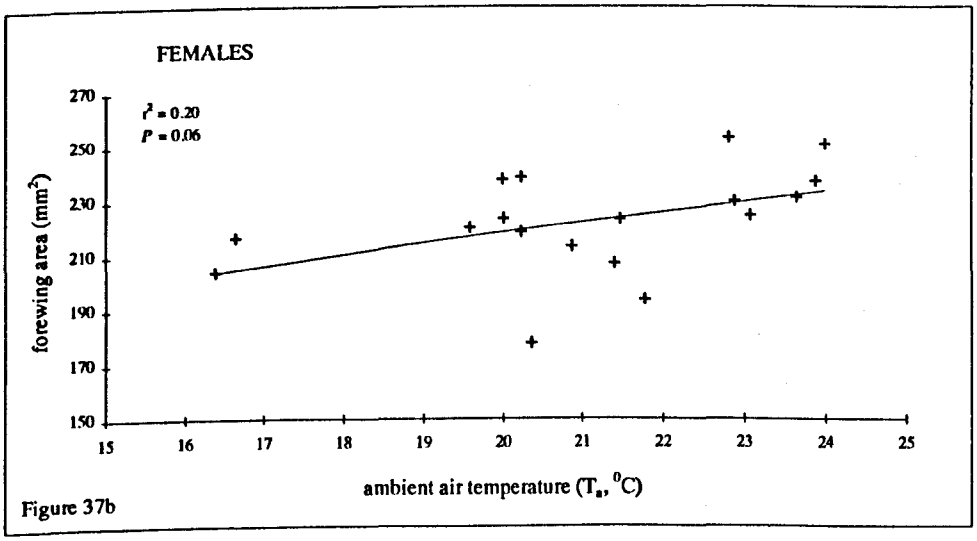


Figure 37b

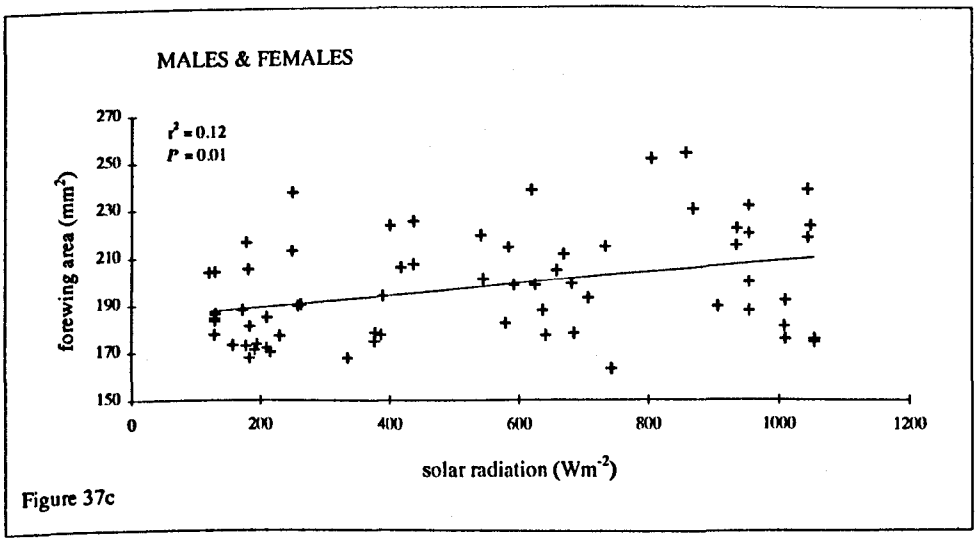


Figure 37c

Figure 37. Area of the doral forewing in relation to ambient air temperature and solar radiation when butterflies were captured. *Maniola jurtina*, southern population.

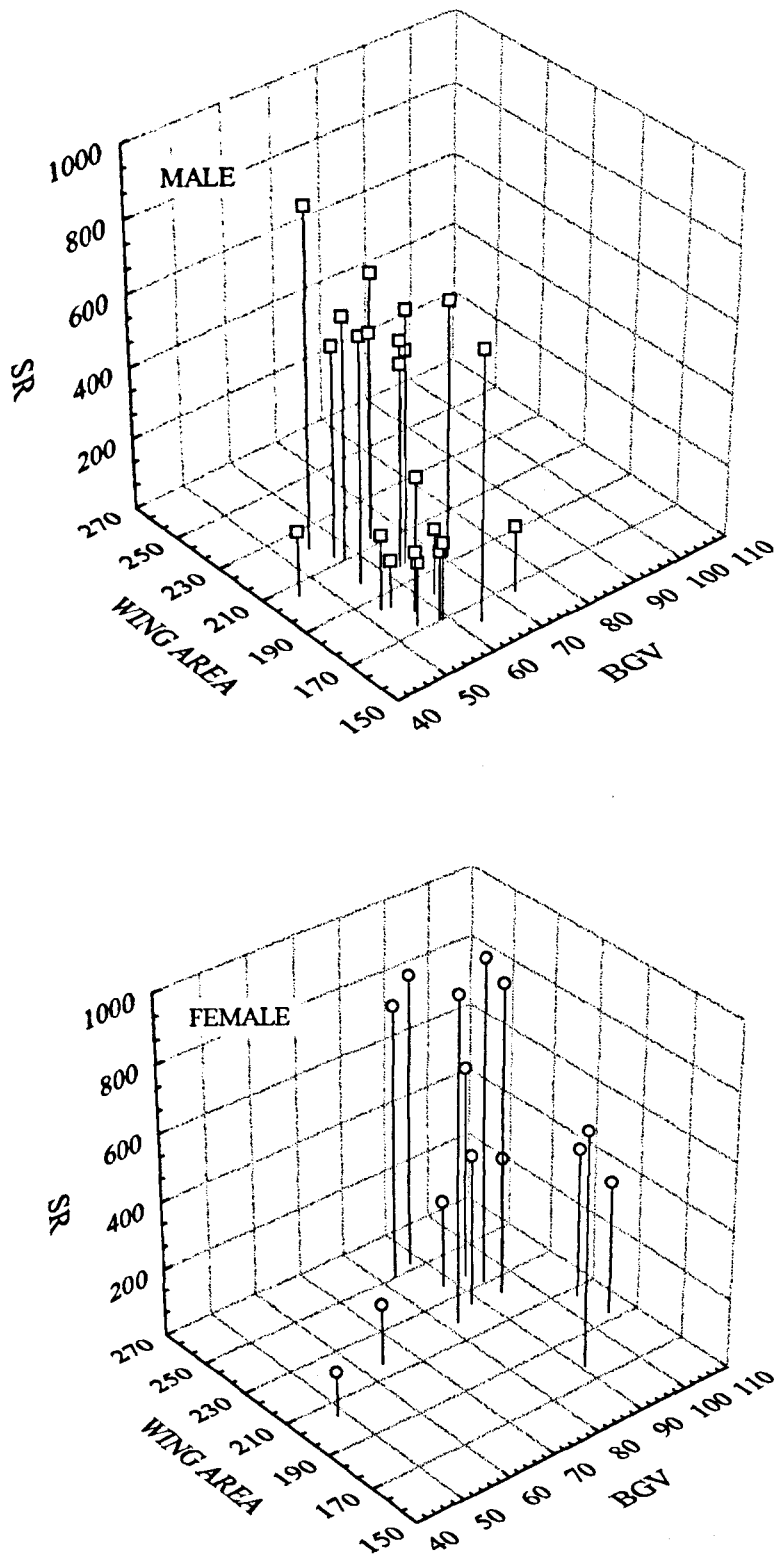


Figure 38. Forewing area (mm^2) & BGV (0=black - 255=white), in relation to SR (Wm^{-2}) at time of capture. Male & female meadow browns, southern population.

13.3.5 Warm-up rates under laboratory conditions

The thoracic temperature of butterflies, placed inside under low light conditions (4 -5 Wm^{-2} were found to be at, or very close to, ambient air temperature. When the butterflies were placed under an artificial light ($\cong$ 80 Wm^{-2}), T_{th} rose at a greater rate than the bare control probe. When the light was switched off, T_{th} gradually returned to T_a levels. Figure 39 demonstrates a typical pattern of thoracic temperature change, under artificial light, for an individual butterfly.

Experiments conducted under laboratory conditions confirmed that, when placed in a dorsal basking position under artificial light, there is a significant negative correlation between BGV and warm-up rate, i.e. butterflies with a darker basal forewing area (lower BGV) warmed-up at a faster rate than those with a paler basal wing area (Figure 40). When males and females are analysed separately the correlation between BGV and warm-up rate does not reach statistical significance. When data for males and females are pooled, the relationship between BGV and warm-up rate is significant ($r^2 = 0.12$, $P = 0.02$).

Although there is no significant difference between the mean warm-up rates of male and female butterflies in this study, the relationship between BGV and warm-up rate makes it likely that males (which are the darker sex) warm-up faster than females. No significant difference was found between mean warm-up rates of southern and northern butterflies, but it is again likely that the darker northern butterflies warm-up faster than the paler southern butterflies.

Again, a larger sample size might help to clarify whether this apparent correlation between BGV and warm-up rate remains robust when the sexes and regions are analysed independently.

No significant relationship was found between warm-up rates and wing size under laboratory conditions.

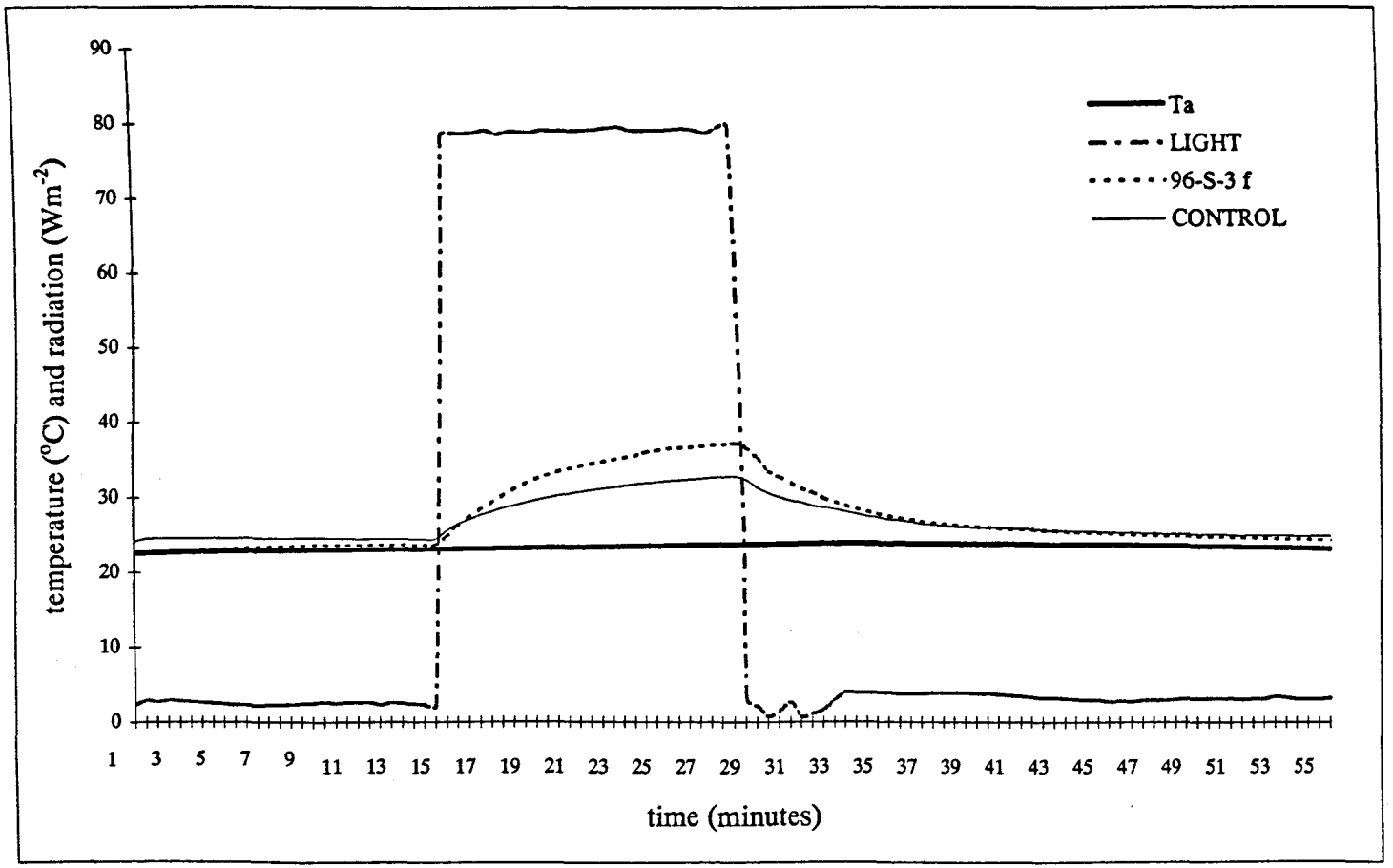


Figure 39. Warm-up rate under laboratory conditions (inside, under artificial light): Butterfly thoracic temperature and bare control probe .
(96-S-3 f = individual butterfly code; a Scottish, female *Maniola jurtina*)

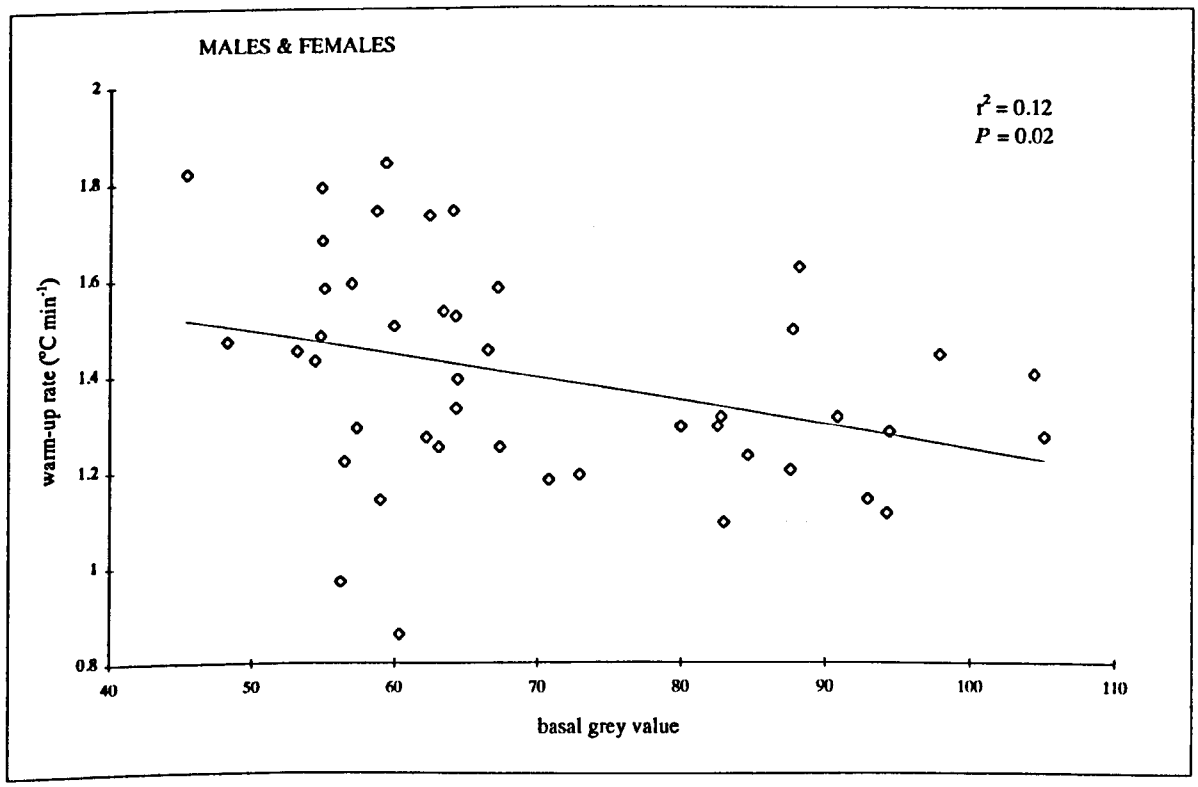


Figure 40. Warm-up rate under artificial conditions in relation to basal grey value of the dorsal forewing, *Maniola jurtina*, southern population.

13.3.6 Warm-up rates in relation to the environmental conditions at the time of capture

Warm-up rates are plotted against SR and T_a at the time of capture, to see whether warm-up rates under artificial light support the evidence from field data. There is a significant negative correlation between SR at the time of capture and warm-up rate under artificial light, for both female ($r^2 = 0.46, P = 0.003$) and male ($r^2 = 0.14$, $P = 0.05$) butterflies. This confirms that butterflies which were active under conditions of low solar radiation warm-up faster than those caught at higher levels of SR (Figures 41a and 41b). When warm-up rates under artificial light are plotted against ambient air temperature, then there is no significant correlation when the sexes are treated independently. When data for males and females are combined there is a weak negative correlation between warm-up rate and T_a at the time of capture ($r^2 = 0.09, P = 0.04$), i.e. butterflies caught in cool conditions tend to warm-up faster than those caught when the air temperature is higher (Figure 41c).

13.4 Discussion

The key results here are that *M. jurtina* from the northern population tend to be larger and darker than those from the south. Data from the southern population support the observation that smaller, darker individuals are more active than larger, paler individuals under conditions of low T_a and SR. Results of laboratory analyses of warm-up rates are consistent with hypothesis that darker individuals warm-up faster. These results give a strong indication that within a population, individuals with different wing morphologies are likely to be active under different weather conditions. It also indicates that the increased melanisation (males and females) and increased size (males) of the northern butterflies is likely to be an adaptation to the generally cooler, cloudier conditions in north west Scotland, allowing the butterflies to reach threshold temperatures for flight at lower T_a and SR than those in the southern population and possibly maintain the required T_{th} for longer.

The finding of increased melanisation in the northern population is consistent with data from other species, where populations living at higher altitude or latitude tend to be

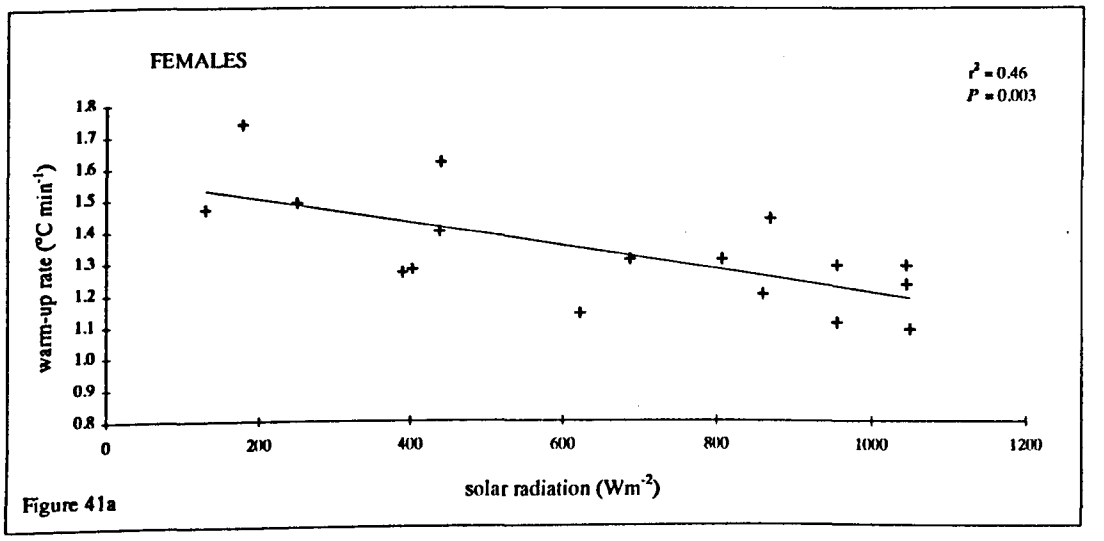


Figure 41a

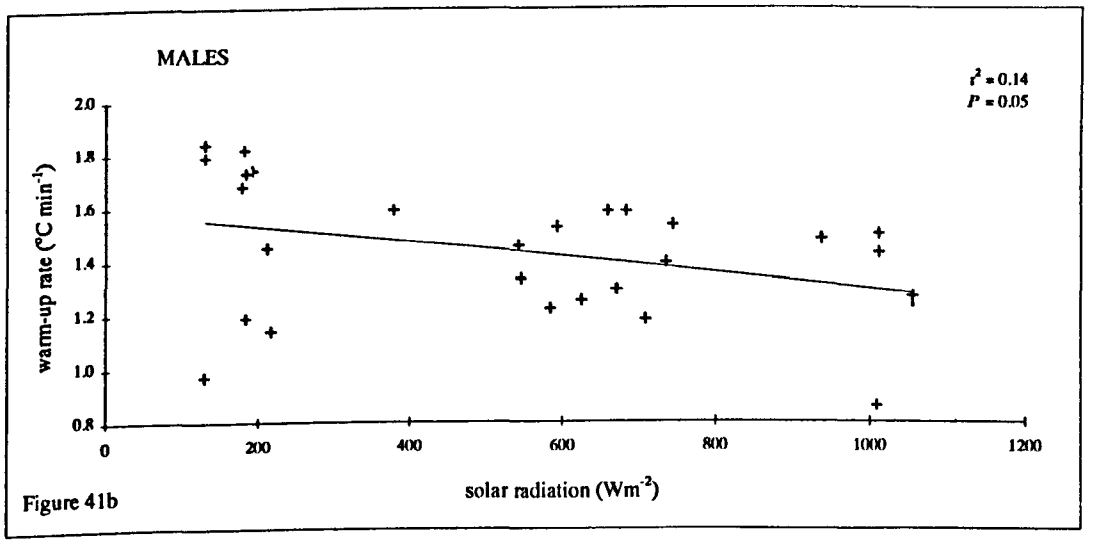


Figure 41b

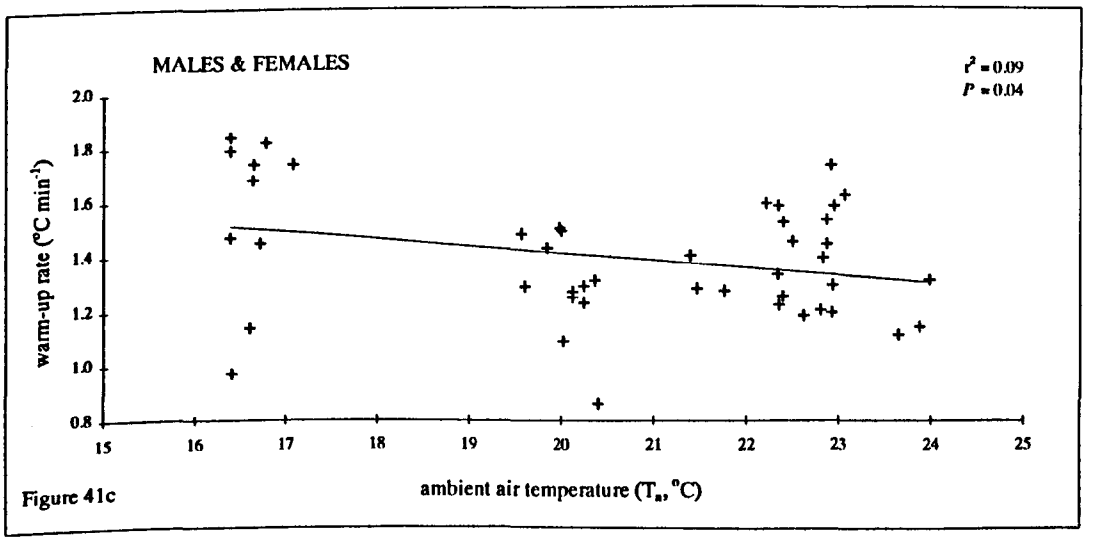


Figure 41c

Figure 41. Warm-up rate under artificial conditions in relation to SR and T_a at time of capture. *Maniola jurtina*, southern population.

darker, with increased thermoregulatory efficiency at low T_a and SR intensity. The adaptive significance of this is through increased flight time at low T_a and SR, and hence time for locating mates, egg-laying and feeding. The proximal mechanism for increased melanisation appears to be controlled by both photoperiod (shorter day length leads to darker individuals) and temperature during developmental (particularly pupal) stages, with lower temperatures leading to darker adults. (Watt 1968, 1969, Douglas & Grula 1978, Roland 1982, Kingsolver & Watt 1983, 1984, Guppy 1986a, 1986b).

Data on melanisation are in line with both theoretical predictions and work on other species (particularly work done in N. America - not all British species increase in melanisation towards the north) but the data on wing size are less clear cut, although they do confirm Goulson's (1991) finding that smaller meadow browns tend to be more active at lower T_a.

For univoltine species in Europe, the general trend is for increased size at lower latitudes, i.e. size *decreases* towards the north (Roff 1980, Nylin & Svard 1991). This trend is generally attributed to the increased growth season and hence increased development time towards the south. In the British Isles, across the full range of species, there is no clear trend for either increase or decrease in size towards the north (Dennis & Shreeve 1989). Dennis & Shreeve (1989) argue that overall wing morphology is a compromise between the requirements of thermoregulation, mate attraction and predator avoidance, which will vary with climatic conditions, habitat structure and behaviour of the individuals.

Adult size may be partially determined by selection on the imago, but the relationship with weather conditions is more complex than for melanisation. Larger butterflies take longer to warm up, but once threshold temperatures for flight have been reached, they can maintain a higher body temperature for longer than smaller individuals, at low T_a, and so can fly for longer (Heinrich 1986b). Whether there is a selective advantage, in terms of thermoregulation, to being large may depend on the degree of variation in weather conditions, as well as overall mean values of T_a and SR. Other advantages of

being large include higher fecundity and longer life expectancy (Stern & Smith 1960, Wickman & Karlsson 1987, Gottard *et al.* 1994, Karlsson *et al.* 1997).

Size is also influenced by growth rate and development time of the larval and pupal stages. Growth rate and development time are both influenced by photoperiod, which appears to have a strong genetic element, and temperature which has a strong environmental effect (Pullin 1986, Nylin *et al.* 1989). However, there is no straight-forward trade-off between development time and size, as growth rate and development time may vary independently, both showing a large degree of plasticity (Nylin 1992, Nylin *et al.* 1993, Gottard *et al.* 1994).

Here, there appears to be an adaptive advantage for increased melanisation of northern butterflies, in terms of thermoregulatory efficiency at low T_a and SR. The adaptive advantage of increased size for northern *M. jurtina* could be through maintenance of required body temperature during flight, which would tie in with the longer flight durations of northern males. However, determinants of, and constraints on final adult size are complex, so it would be unwise to generalise from these results.

It could be argued that since degree of melanization and size might both be under environmental control, that they cannot strictly be described as "adaptations". However, as the relationship between phenotypic and genetic variation is not necessarily clear cut (Shapiro 1976), there may be an adaptive advantage in a genotype having the ability to produce a range of phenotypic forms - which are advantageous under different climatic conditions (e.g. Douglas & Grula 1978). For a more detailed discussion of genotypic and phenotypic variation, see Chapter 2, section 2.2.4.

CHAPTER 14. SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

The primary aims of this research were:

1. To determine the specific effects of temperature, solar radiation and wind speed on the behaviour of meadow browns.
2. To investigate whether there are differences in behaviour between male and female butterflies and between different geographic/climatic areas.
3. To determine whether variation in wing morphology within a population and between populations in different geographic/climatic areas is adaptive in terms of thermoregulatory efficiency.

The research was carried out in the context of an increasing interest in the influence of climate and weather on butterfly behaviour, distribution, and abundance, and how marginal and core populations may differ, particularly in aspects of their behaviour and morphology which may affect reproductive success.

The results for each section of the research have been discussed in the preceding chapters, so here I summarise the main findings, in the context of the original aims.

14.1 Effects of temperature, solar radiation and wind speed on behaviour

To investigate the specific effects of meteorological variables on behaviour, I recorded these data on a time scale appropriate to the changes in butterfly behaviour (60 s intervals), in order to obtain detailed information about behaviour and weather conditions on a "micro-scale".

Active flight

Butterflies must attain thoracic temperatures of approximately 30°C before active flight is possible, so in cool, cloudy conditions, raising thoracic temperatures to this level will be more difficult to achieve. In Chapter 6, which gives an overview of the influence of weather on behaviour at the population level, it is shown that at low ambient air temperatures and levels of solar radiation (T_a 14 - 20°C, SR > 200 Wm^{-2}) some individuals engage in active flight, but a higher proportion were more likely to exhibit

only short "escape flights," these being in response to disturbance. The clearest demonstration of the effect of weather conditions on active flight at the individual level was for males from the southern population. Mean and maximum flight duration both showed a positive correlation with solar radiation intensity and a negative correlation with wind speed. Maximum flight duration for southern females also showed a positive correlation with solar radiation intensity.

Possible explanations for the lack of correlations between flight duration and meteorological variables for northern butterflies are discussed in Chapter 7, but a strong possibility is that this was an effect of sample size, with a much larger data set for southern males than for other butterflies.

If flight duration is limited by weather conditions, particularly low solar radiation intensity and wind speed, this has implications for survival and reproductive success of individual butterflies. Predation rates have been found to be higher in cool cloudy conditions (Bowers *et al.* 1985, Kingsolver 1987b), which may be due to butterflies being unable sustain active flight. Flight is also essential for locating mates by males, for finding nectar sources, by both sexes, and for locating suitable egg laying sites for females. Kingsolver (1985a) argues that thermoregulation and flight are key aspects of butterfly behaviour which link weather conditions to the population ecology and dynamics of temperate butterflies, through their influence on realised fecundity of individual butterflies.

Alighted behaviour: basking, heat avoiding and intermediate posture

Results from Chapter 9 show that the duration of basking decreased with increasing levels of solar radiation for male butterflies in both southern and northern regions. This suggests that males were basking primarily to raise their thoracic temperature to the level required for active flight. Once this temperature was attained they would cease basking. As solar radiation intensity increased the time needed to achieve the necessary thoracic temperature would decrease. At high T_a and SR butterflies of both sexes in both regions were more likely to adopt a heat avoiding posture to prevent overheating.

Feeding behaviour

The general tendency was for feeding duration to decrease with increasing T_a and SR (Chapter 10). Meadow browns appear to be acting as "time minimizers" (Schoener 1971), maximizing fitness by minimizing the time spent feeding to gather a given energy requirement. At higher temperatures and SR levels feeding may be more efficient as the viscosity of the nectar is likely to be lower and suction through the proboscis more powerful (Pivnick & McNeil 1985). At higher ambient air temperatures and levels of solar radiation, conditions will also be more favourable for other flight related activities, to which the butterflies must allocate a proportion of their time.

Conversely, at low T_a and SR, the feeding time required to gather a given amount of energy will increase, but there may be less conflict between the time allocated to feeding and time allocated to other flight related activities - for which conditions would now be less favourable.

Egg-Laying

Egg-laying was observed in both populations over a wide range of temperatures and SR intensities. In the southern population, as SR increased the time taken to lay an individual egg decreased, as did the time taken to walk between laying individual eggs during any egg-laying sequence (Chapter 11). This could be due to a generally increased metabolic rate in warmer, sunnier conditions. In other species, low temperatures have also been shown to decrease the rate of egg maturation and oviposition (Stern & Smith 1960, Boggs 1986).

14.2 Differences in behaviour between male and female butterflies and between different geographic/climatic areas.

Active flight

Male butterflies in both regions flew for significantly longer durations and spent a greater proportion of their time in flight than females. This supports the findings of other work on meadow browns (Pollard 1981, Brakefield 1982a) and reflects the different functions of flight for the sexes. Flight by males was primarily to patrol in

search of females, interspersed with visits to nectar plants. Females flew in order to feed or lay eggs and spent the rest of the time alighted low down in the vegetation.

There was no significant difference in flight duration for females in the southern and northern regions. This probably reflects the fact that the resources for which flights were undertaken were at a similar level of availability in both regions - i.e. females in both populations had to fly for similar distances and durations to locate nectar sources and suitable egg-laying sites within their habitats. This was in contrast to the much longer flight durations found for northern males compared to southern males. The functional requirement for them do so was to increase their chances of finding receptive females, which were present at a much lower density in the north than the south.

It could be argued that there might be an advantage for southern males also flying for longer, to increase the rate at which they encountered receptive females. The fact that they do not fly for the same duration as northern males suggests that there may be some costs involved. I suggest that the cost of increased flight duration may be in decreased life span (through faster depletion of energy reserves and increased wing deterioration, Brakefield 1982b, Warren 1992). In the north, flight seasons are shorter and emergence tends to be more synchronised (Brakefield 1987), so the cost of decreased life span is off set by the benefit of increased chance of finding receptive females over a shorter period. In the south, where emergence is spread over a longer period, the balance would be in favour of a longer life span. A similar argument is used by Gottard *et al.* 1994, is relation to differences in life span of male speckled woods in Sweden and Madiera.

Interactions with other butterflies

Results from Chapter 8 demonstrate that in both regions the most frequently recorded encounters were of male meadow browns chasing another male meadow brown. These encounters were of longer duration than those with other species of butterfly. The high proportion of time spent by males in flight may mean that patrolling is the predominant mate location activity. However, in the southern population, males appeared to be using both patrolling and perching, with chases being initiated by settled males.

This difference in behaviour between males in the two regions may again be influenced by the different population densities and spatial distribution of resources (including females) in the two regions. In the north, the habitat patches occupied by the meadow browns are small compared to those in the south. Northern males may increase their chances of finding receptive females by a combination of patrolling intensively, and opportunistically investigating any potential mates which are encountered while engaged in other activities. In the south, habitat patches are more extensive, so females, although more abundant than in the north, are likely to be dispersed over a wider area. In these circumstances a combination of perching and patrolling may offer the optimum strategy.

Alighted behaviour: basking, heat avoiding and intermediate posture

In Chapter 9, the clearest result relating to differences between the regions (and between southern males and females) was the greater height at which southern males were found alighted in the vegetation, compared to either southern females or northern butterflies of both sexes. One explanation for this could be the greater use of perching as a mate location strategy by southern compared to northern males, with the greater height increasing the distance over which potential mates could be detected.

Data from Chapter 6 shows that, overall, females spent a higher proportion of their time alighted than did males, most frequently adopting an intermediate posture (neither basking nor heat avoiding). The higher proportion of time that females spent alighted, rather than engaged in more active behaviour, again reflects the different behavioural ecology of the sexes, with males flying primary to locate mates, whereas females, once mated, will usually try and avoid males by remaining alighted in the vegetation.

The function of basking to raise thoracic temperature may differ between the sexes. Once the required thoracic temperature has been reached, males will usually fly (hence the decreased basking duration with increased levels of SR). Females may need to try and keep thoracic temperature in the range where flight is possible (to feed, lay eggs or escape predators) but they do not fly as readily as males, so basking duration is less closely coupled with SR intensity. In addition, females may try to maintain a high

thoracic temperature to increase the rate of egg maturation (Stern & Smith 1960), rather than just in readiness for flight.

Feeding behaviour

There were no significant differences between the regions or between the sexes in the amount of time allocated to feeding. Other studies (Brakefield 1982a, Pivnick & McNeil 1985) have found that females tend to feed more frequently and for longer duration than males, but the data from this study did not confirm this. Southern females tended to allocate more time to feeding than males (although the difference was not significant), but in the northern population the reverse was the case.

Egg-laying

A model based on the amount of time allocated to egg-laying, the rate of egg production and the density of meadow browns in each region, indicates that the realised fecundity of northern females is lower than that of southern females, and that far fewer eggs are laid per unit area of habitat in the northern population. This is almost certainly one of the main factors contributing to, and maintaining, a low population density in the north.

14.3 Variation in wing morphology within populations and between populations in different geographic/climatic areas

Meadow browns from the northern population tended to be larger and darker than those from the south (Chapter 13). Under conditions of low T_a and SR, smaller, darker individuals tended to be more active than larger paler individuals. Darker butterflies also warmed up faster under laboratory conditions.

The finding of increased thermoregulatory efficiency of darker meadow browns, at low ambient air temperatures and solar radiation intensity, is in accordance with the work on other species of butterflies, such as that of Watt (1968, 1969) on *Colias*. Goulson (1991) also found that smaller meadow browns tend to be more active at lower ambient air temperatures.

Within a population, variation in wing morphology, relating to efficiency of absorption of solar radiation and thermal stability, is likely to be maintained by short term variation in weather conditions. Different sub-sets of the population are likely to be active under different weather conditions, so although smaller, darker butterflies are likely to be at an advantage in cool, cloudy conditions, they may be in danger of overheating in hot sunny conditions - when paler butterflies may be able to remain more active.

Adaptations to increase thermoregulatory efficiency are likely to be of prime importance in populations living in marginal weather conditions where time available for flight is more limited. Although there was no difference between northern and southern butterflies in the thoracic temperature associated with active flight (Chapter 12), the increased melanization of butterflies in the northern population will allow them to achieve thoracic temperatures required for flight at lower T_a and SR levels, and so increase the time available for flight related activities. The larger size may also confer greater thermal stability, allowing them to remain active for longer periods (Heinrich 1993).

14.4 Sequences of behaviour

In the previous chapters I have taken each category of behaviour and analysed it independently to determine the influence of environmental variables, particularly temperature and solar radiation, on that behaviour. Within this framework comparisons can then be made between males and females and between southern and northern populations. The disadvantage of using this approach to analysis of behaviour is that it is easy to lose sight of the overall picture. Each separate activity obviously does not occur in isolation, but as part of a continuous sequence of behaviour, the characteristic pattern of which may vary between the sexes and between the regions.

Here I attempt to restore the balance by giving examples of "typical" sequences of behaviour, plotted over real time, for five individual butterflies (after Dennis & Williams 1987). Figure 42 gives sequences of behaviour for two southern females (one engaged in egg-laying, the other non egg-laying), a southern male, a northern male and a northern female. All sequences are for a ten minute period, when weather conditions

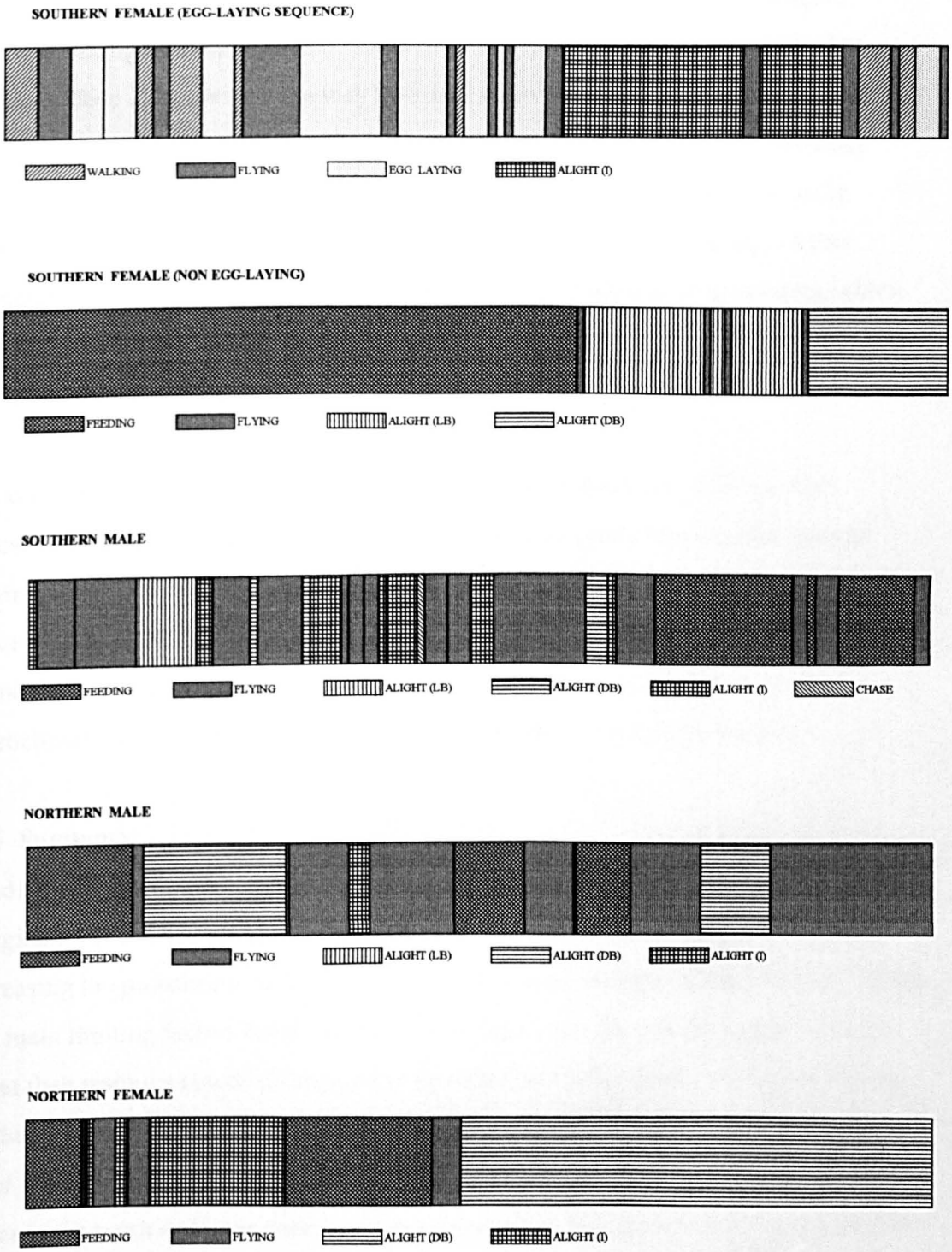


Figure 42. Sequences of behaviour in *M. jurtina*, over ten minute periods.

ALIGHT (DB) = DORSAL BASKING
ALIGHT (LB) = LATERAL BASKING
ALIGHT (I) = INTERMEDIATE POSTURE

were warm and sunny (T_a of 20-21°C, SR of 500-600 W m^{-2}). These plots again illustrate the higher frequency and longer duration of flights in males compared to females. They also illustrate the way that males in particular are behaving as what Dreisig (1995) describes as true shuttling ectotherms, alternating between basking (heating) and activity (flying or feeding). As T_{th} will be increasing or decreasing according to the behaviour in which any butterfly is engaged, Dreisig argues that temperature is acting as a constraint on achieving preferred body temperatures, which necessitates basking, and time spent basking is a constraint on time allocated to other activities (flying and feeding).

The end result is that butterflies shuttle between these behaviours, with the time allocated to each depending on their physiological state (body temperature, energy requirements etc.) and external conditions (temperature, SR, presence of other butterflies etc.). No heat avoiding behaviour is included in these sequences, but at high T_a and SR the necessity of adopting a heat avoiding posture or seeking a cooler microclimate will also place constraints on time available for other activities.

14.5 Summary

Conditions in north west Scotland can be described as marginal for the meadow brown. Marginality is defined as "the impact that environment and landscape factors have in decreasing the probability of population survival and persistence" (Shreeve *et al.* 1996). The main limiting factors for the northern population are likely to be weather related rather than resource based. Compared to populations further south, conditions for the northern population are cooler, with fewer hours of sunshine, and windier and rainier - all of which will limit activity time. Density of meadow browns was found to be much lower in the north and their habitat use more restricted, being confined to south-facing slopes. At the population level, realised fecundity was also substantially lower.

Under these conditions individuals are expected to show adaptations to increase thermal efficiency. Northern meadow browns were found to be larger (males) and show

increased basal melanization (males and females). Darker individuals were shown to be more active than paler ones under conditions of low ambient temperature and solar radiation, and to warm-up faster under laboratory conditions. The increased size of the northern meadow browns may enable them to maintain higher thoracic temperatures during flight and so increase flight duration. Although this was not demonstrated directly here, the longer flight duration of northern males indicates that this is a strong possibility.

The variation in morphology both within and between populations, which allows different individuals to be active under different weather conditions, is probably one of the factors which contributes towards the success of meadow browns as a species. I suggest that another factor may be their behavioural flexibility. Examples of this, demonstrated in this research, include the use of both patrolling and perching mate location behaviour, the relative frequency of which may depend on the factors such as habitat and density of females. Another example of behavioural flexibility is the use of both dorsal and lateral basking postures.

One interesting finding to emerge from this research is that males in the northern and southern regions appear to be more dissimilar, in terms of behaviour (e.g. in flight behaviour and mate location behaviour) than females. This may be due to the different behavioural ecology of the two sexes, with males being substantially more "action-based" than females, and activity-based behaviour (particularly flight related activities) having greater sensitivity to meteorological variation than the more sedentary behaviour of the females.

Despite their behavioural flexibility and the adaptations for increased thermal efficiency, the northern population can still be seen as "making the best of a bad lot". The low density, decreased fecundity, restriction to more localised habitat patches and reduced flight period, all serve to make northern populations more vulnerable to periods of bad weather (Thomas *et al.* 1994). The population dynamics are also likely to differ from those in the south, with the smaller, more isolated populations being more prone to local extinction through environmental or demographic stochasticity (Gilpin 1987).

Although the meadow brown appears to be a robust, successful species, it is not immune from factors such as changes in climate or habitat (e.g. the replacement of fine leafed grasses with coarser species can lead to a decline in numbers, Pollard *et al.* 1998). Populations at the edge of their range are likely to be the most vulnerable.

14.6 Assumptions and limitations of the research

In this study I have referred to, and made comparisons between, the southern and northern "populations". However, field work was conducted at three different sites in the south, and two different sites in the north. The butterflies at the sites within each region were probably, in reality, separate populations, which may or may not have been linked as a metapopulation. I made the assumption that the behaviour and morphology of the butterflies at the separate sites within each region was likely to be more similar to that of the butterflies at other sites within the region, than it was to any site from the other geographic area. If this assumption was true, then the sites within each region could be treated together for the purposes of this research.

Between site variation within each region makes an interesting study in its own right (e.g. Goulson 1991), especially in the northern region where there may be greater between-population variation (Shreeve *et al.* 1996).

One of the major limitations of this research was the small sample size of the northern population. This was due to the low density of butterflies in the area and the short flight season, limiting time available for observations. A larger sample size would obviously be advantageous in detecting "weak" trends, which do not show up in small samples, and for increasing the reliability of the data. Although more prolonged and intensive behavioural observations would be advantageous, I would be concerned about taking a large destructive sample (e.g. for wing morphology analysis), as the low population density means that the impact on the population would be greater in the north than in the higher density southern sites.

14.7 Suggestions for future research

Dorsal & Lateral Basking

As meadow browns have been demonstrated to use both dorsal and lateral basking, further analysis of this could include a comparison of warm-up rates of butterflies placed in a dorsal or lateral posture, to determine whether there was any difference in thermoregulatory efficiency between the two postures (Heinrich 1986b found that there was no difference in warm-up rates of *A. glandon* placed in dorsal or lateral basking postures). This could be combined with image analysis of the ventral hindwing, to determine whether there was any difference in the degree of basal melanization between northern and southern populations.

Furriness

Increase in pubescence or furriness has been demonstrated in populations of butterflies living in cooler, windier conditions (e.g. Kingsolver & Moffat 1982). A comparison between furriness of northern and southern butterflies would make a useful addition to the information on morphological adaptations found in the northern population.

Enzymes

Northern male meadow browns where able to sustain flight for longer duration than southern males. In *Colias,* variation in PGI allozyme frequency was found to be associated with altitude, weather and flight activity (Watt 1977, 1983, Watt *et al.* 1983, 1985). Goulson (1993) found that variation in PGM allozyme frequencies in the meadow brown was associated with the ability to sustain prolonged flight. Future research could include a comparison of allozyme frequency in the two populations, to determine whether this was a contributory factor to the difference in flight duration.

Phenotypic and Genetic Variance

Marginal, more spatially isolated populations at edge of a species range may be subject to decreased within-population genetic variance, if isolation and founder effects are important. If migration between populations is low, then between-population differentiation may be increased (Shreeve *et al.* 1996). Morphological variation can be accounted for by both phenotypic plasticity (response of larval and pupal stages to

temperature and photoperiod) and genetic variation. These are not mutually exclusive since the degree of phenotypic plasticity which can be expressed may be under genetic control (Shapiro 1976, Dennis & Shreeve 1989). If there is increased selection for thermoregulatory efficiency at higher latitudes, then traits such as increased melanization, which increase thermoregulatory efficiency, might be expected to show less variation in northern than in southern populations. If this was the case then increased selection might contribute to the decreased within-population variance which is predicted for marginal populations at the northern edge of their range (see above). However, increased selection for thermoregulatory efficiency could also lead to similar adaptations in isolated populations, experiencing similar climatic selective pressures, thus *decreasing* between population differentiation in the relevant traits.

The capacity for producing morphological variants adapted to local conditions may be a key factor determining whether a species can expand its range into new geographic/climatic areas (Douglas & Grula 1978) or maintain its range under changed climatic conditions, such as global warming. Unravelling the extent of genetic and phenotypic influences on variation in wing morphology in meadow browns would contribute to a greater understanding of factors influencing their success as a widespread, abundant species, and the limitations on populations at the edge of their range.

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